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ABSTRACT

We evaluated the effects of different oxidation levels of lipids on protein degradation and biogenic amines (BAs) formation during Sichuan-style sausages processing. Lipids with varying degrees of oxidation were obtained through storage at different temperatures and added as raw materials of Sichuan-style sausages, followed by the analyses of lipid oxidation, protein degradation, biogenic amine content, and other indicators. During the processing, with increasing degree of lipid oxidation, the contents of peroxide value (POV), thiobarbituric acid reactive substances (TBARs), protein degradation index (PI), amino acid nitrogen (AAN), free amino acids (FAAs), and BAs increased. Based on the protein electrophoresis results, the higher the oxidation degree of pig backfat, the higher degree of sarcoplasmic protein oxidation, and the greater myofibril protein degradation. Pearson correlation revealed that lipid oxidation, protein degradation, and BAs content correlated significantly ($P < 0.05$).

1. Introduction

Sichuan-style sausage is loved by consumers, primarily because of its spicy taste. During the ripening process of Sichuan-style sausage, a series of chemical reactions occur in its composition, such as lipid oxidation and protein degradation, which significantly contribute to its unique flavour and taste (Corral, Salvador, Belloch, & Flores, 2014).

In meat products, lipid oxidation mainly occurs as a series of oxidation reactions of free amino acids to form aldehydes, alcohols, ketones, acids, hydrocarbons, furans, and other oxidation products (Chen et al., 2018; Cheng, Sørensen, Engelsen, Sun, & Pu, 2019; Papuc, Goran, Predescu, & Nicorescu, 2017). Moderate lipid oxidation gives the product an excellent colour and a pleasant flavour, although excessive lipid oxidation can affect the product's texture, colour, and flavour. Excessive oxidation generates several active free radicals, which endangers consumers' health and decreases the edible value (Falowo, Fayemi, & Muchenje, 2014). Several studies have demonstrated that lipid oxidation in meat products can induce protein oxidation (Wang, He, Emara, Gan, & Li, 2019; Wen et al., 2019). A few literature reports about the effect of lipid oxidation on protein degradation. Lipid oxidation and protein degradation are critical chemical reactions that affect the quality of fermented sausages (Flores & Piornos, 2021). Therefore, the study of the relationship between lipid oxidation and protein degradation in fermented sausages is critical.

Sichuan-style sausage is rich in protein and lipids. The product has an excellent sensory quality, and it produces a variety of potentially toxic substances, such as heterocyclic amines and biogenic amines (BAs) (Yang, Sun, & Pan, 2018; Liu et al., 2022). BAs are typical active components in the organism and play an essential physiological role, but excessively intake of BA endangers human health (Jia et al., 2020). The relationship between lipid oxidation and BA formation is rarely reported in the literature, and most of them are simulation experiments. A few studies have conducted more systematic research on its relationship; for instance, in 2010, Hidalgo et al. (Hidalgo, Delgado, Navarro, & Zamora, 2010) reported that the decarboxylation reaction of amino acids not only occurred under the action of enzymes but also through the chemical pathways in the presence of carbonyl compounds, however, the study was conducted under the condition of simulating a high-temperature reaction of acrylamide formation in heat-treated foods. In addition, in 2012, Zamora et al. (Zamora, Delgado, & Hidalgo, 2012) reported that

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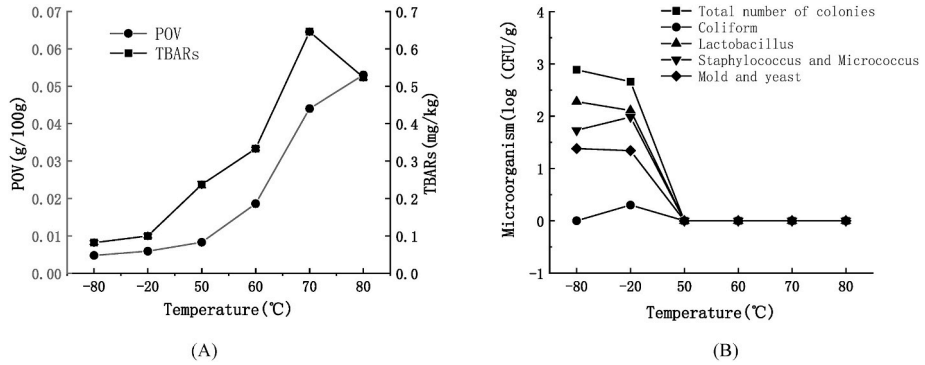


Fig. 1. POV, TBARs and microorganisms of pig backfat under different temperature treatments.

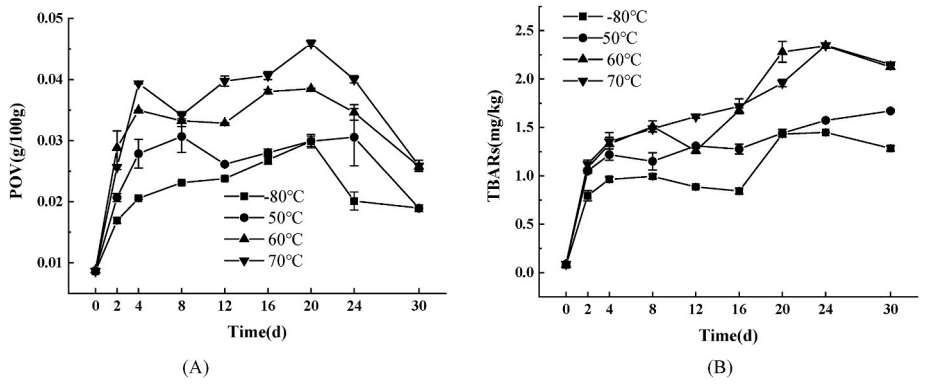


Fig. 2. Effect of oxidised pig backfat on POV and TBARs in sausage.

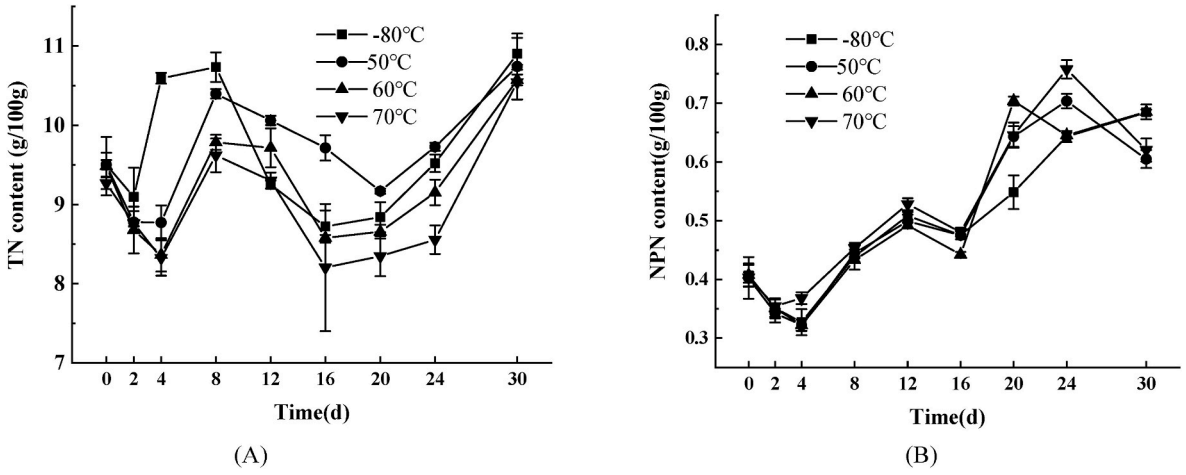


Fig. 3. Effect of oxidised pig backfat on TN and NPN in sausage.

phenylalanine could decarboxylate to produce β-phenylethylamine in the presence of active carbonyl compounds, a standard lipid oxidation product present in food under mild conditions. In 2013, Hidalgo et al. (Hidalgo, Navarro, Delgado, & Zamora, 2013) studied the reaction of histidine decarboxylation to form histamine in the presence of lipid oxidation products. In 2016, Hidalgo et al. (Hidalgo, León, & Zamora, 2016) studied the effect of second amino acids on the decarboxylation of phenylalanine to phenylethylamine induced by lipid derivative-active carbonyl, and the result demonstrated that adding a second amino acid usually promoted the formation of BA in the system. These simulation experiments revealed that the lipid oxidation products could promote the formation of BA. However, meat product is complex system with several chemical changes occurring during their processing and storage, the effect of lipid oxidation on BA formation in meat products requires further study.

In this study, pig backfat with different oxidation levels were obtained through high-temperature treatment and then mixed with lean pork as raw material to prepare Sichuan-style sausage. Some indices of lipid oxidation, protein degradation, and BA during the processing were determined, and the relationship among lipid oxidation, protein degradation, and BA formation during maturation of Sichuan-style sausage was analysed.

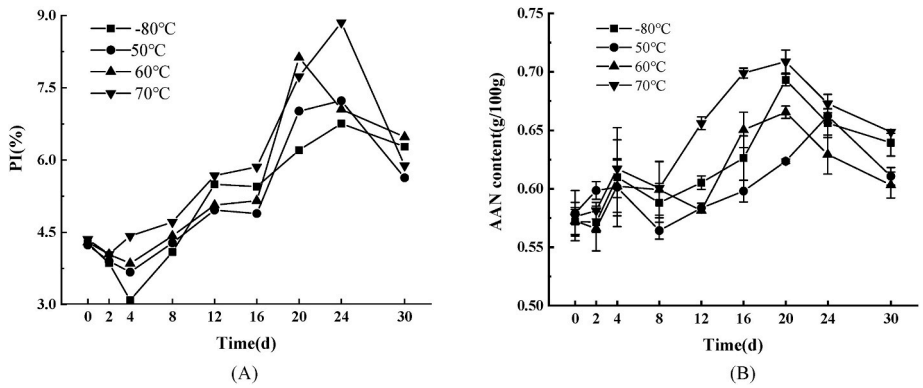


Fig. 4. Effect of oxidised pig backfat on PI and AAN in sausage.

Table 1
The effect of oxidised pig backfat on FAA content in sausage.

FAA	The 30th day of fermentation maturity(g/100g)			
	−80 °C	50 °C	60 °C	70 °C
Aspartic acid	0.03 ± 0.00^{a}	0.04 ± 0.00^{a}	0.06 ± 0.00^{b}	0.09 ± 0.00^{c}
Threonine	0.08 ± 0.01^{a}	0.10 ± 0.01^{ab}	0.11 ± 0.00^{b}	0.14 ± 0.00^{c}
Serine	0.06 ± 0.00^{a}	0.09 ± 0.00^{b}	0.10 ± 0.00^{b}	0.14 ± 0.00^{c}
Glutamate	0.48 ± 0.01^{a}	0.48 ± 0.02^{a}	0.76 ± 0.02^{b}	0.97 ± 0.03^{c}
Glycine	0.05 ± 0.00^{a}	0.06 ± 0.00^{a}	0.06 ± 0.00^{a}	0.09 ± 0.00^{b}
Alanine	0.16 ± 0.01^{a}	0.23 ± 0.02^{b}	0.24 ± 0.00^{b}	0.31 ± 0.01^{c}
Cystine	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}
Valine	0.08 ± 0.00^{a}	0.10 ± 0.00^{b}	0.11 ± 0.00^{b}	0.14 ± 0.00^{c}
Methionine	0.03 ± 0.00^{a}	0.04 ± 0.00^{a}	0.05 ± 0.00^{a}	0.07 ± 0.00^{b}
Isoleucine	0.06 ± 0.00^{a}	0.07 ± 0.00^{a}	0.08 ± 0.00^{a}	0.10 ± 0.00^{b}
Leucine	0.14 ± 0.00^{a}	0.16 ± 0.01^{ab}	0.19 ± 0.00^{b}	0.26 ± 0.02^{c}
Tyrosine	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}	0.05 ± 0.00^{b}	0.05 ± 0.00^{b}
Phenylalanine	0.08 ± 0.00^{a}	0.10 ± 0.00^{b}	0.13 ± 0.00^{c}	0.16 ± 0.00^{d}
Lysine	0.10 ± 0.00^{a}	0.13 ± 0.00^{b}	0.14 ± 0.00^{b}	0.19 ± 0.01^{c}
Ammonia	0.08 ± 0.00^{a}	0.13 ± 0.00^{c}	0.11 ± 0.00^{b}	0.17 ± 0.00^{d}
Histidine	0.03 ± 0.00^{a}	0.04 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}
Arginine	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Proline	0.08 ± 0.00^{a}	0.10 ± 0.00^{a}	0.08 ± 0.00^{a}	0.10 ± 0.00^{a}
Total Amino Acids	1.49 ± 0.03^{a}	2.09 ± 0.06^{b}	2.25 ± 0.02^{b}	2.88 ± 0.09^{c}

Different letters (a-f) within a row indicate significant differences among the different treatments ($P < 0.05$).

2. Materials and methods

2.1. Preparation of pig backfat with different oxidation degrees

The whole pig backfat was purchased from the local retail market (Ya'an, China). The surface fat (approximately 0.2 cm) of the entire pig backfat was removed, and the remaining portions were trimmed to achieve a dimension of 3 × 2 × 0.2 cm^3 (length/wide/height) and packed in sterile homogenised bags. Each fillet weighed approximately 200 g. The portions were incubated at −80 °C, −20 °C, 50 °C, 60 °C, 70 °C, and 80 °C for 6 h and subjected to oxidation. Then, the POV and TBARs values of pig backfat were determined. Meanwhile, the total number of colonies was determined on the PCA medium, the number of fungi and yeasts was enumerated on the PDA medium, the number of *Lactobacillus* was determined on the MRS medium, and the number of *Escherichia coli* was listed on the VRBA medium.

2.2. Production of Sichuan-style sausage

The pork backfat with significant lipid oxidation was selected to prepare sausages. The natural sausage was filled with 70 kg of lean pork, 30 kg of backfat, 2.5 kg of salt, 1.5 kg of sugar, 1.0 kg of pepper powder, 1.0 kg of Chinese distilled spirit (Erguotou, Beijing Hongxing Co., Ltd., China), 0.4 kg of pepper powder, 0.1 kg of glucose, 0.05 kg of Shi-sanxiang, and 0.075 kg of nitrate mixture. The prepared sausage was fermented for 2 days at 20 °C under a relative humidity (RH) of 75%, then stored at 13 °C under 60% RH, and matured for 28 days (DHP-9272B, Shanghai Yiheng Scientific Instrument Co., LTD, China). For detection, the samples were taken on days 0, 2, 4, 8, 12, 16, 20, 24, and 30. Every sample was analysed in three replicates.

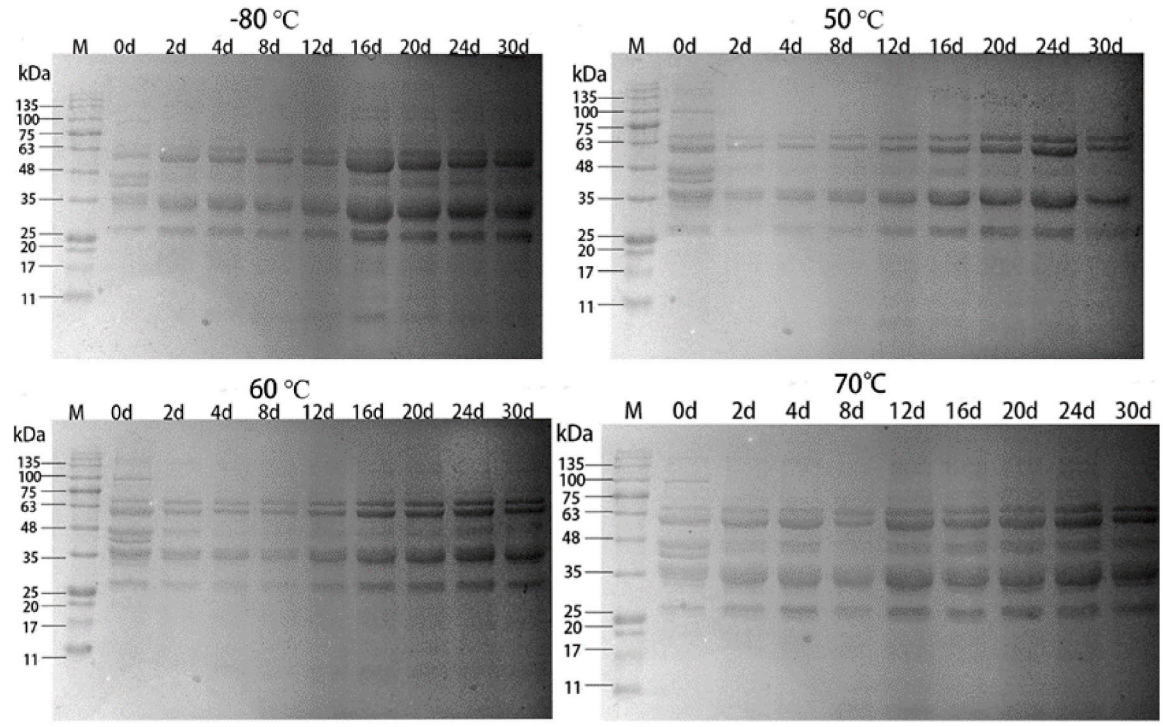
2.3. Lipid oxidation analysis

2.3.1. Determination of the peroxide value

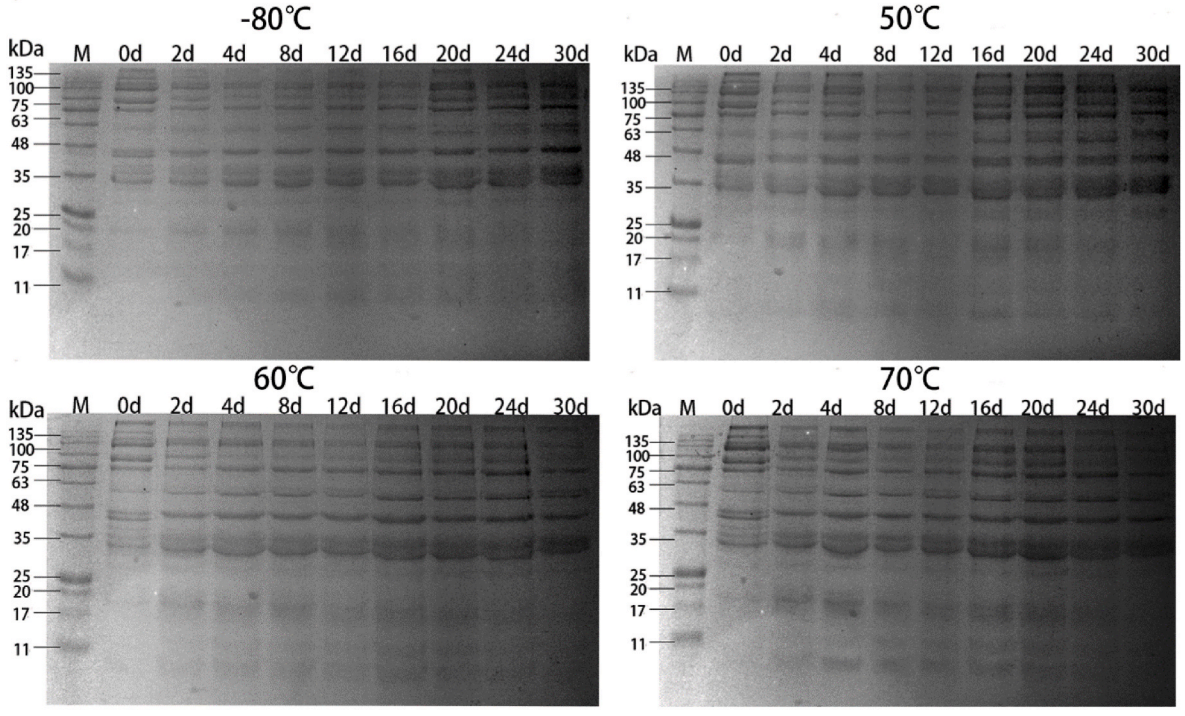
The peroxide value of each sample was measured according to the mothed of ISO 3960:2017. Briefly, 50 g sample was mixed with 150 mL petroleum ether (boiling point of 30°C–60 °C) overnight (37 °C) and then filtered. The filtrate was heated to a constant weight in a water bath <40 °C (XMTD-7000, Beijing Yongguangming Medical Instrument Co. LTD, China). And then, 2 g of this liquid was added to a 30 mL chloroform-glacial acetic acid mixture and 1 mL of saturated potassium iodide solution. The reaction mixture was placed in the dark for 3 min. The reaction mixture was titrated with a solution containing 100 mL of water, 1 mL starch indicator, and 2 mmol/L sodium thiosulfate solution until the blue colour of the reaction mixture disappeared.

2.3.2. TBARS measurement

TBARS was determined by using Vyncke et al. method (Vyncke, 1975). Briefly, 10 g of the sample was homogenised with 50 mL of 7.5g/100 mL TCA-EDTA mixture, filtrated and the obtained filtrate was diluted to 50 mL volume with TCA-EDTA. Then, 5 mL of the solution was incubated with 5 mL of 0.02 mol/L TBA solution at 90 °C for 40 min, and the absorbance was measured at 532 nm.



(A)



(B)

Fig. 5. Effects of oxidised pig backfat on sausage sarcoplasmin and myofibrin

2.4. Protein degradation analysis

2.4.1. Determination of total nitrogen (TN) and non-protein nitrogen (NPN)

TN and NPN were determined by the Kjeldahl method (AOAC, 1990). For TN analysis, 2 g of the minced sausage, 0.4 g $CuSO_4$, 6 g K_2SO_4 and 20 mL H_2SO_4 were added to a digestion tube and digested at 350 °C for 2 h, and then total nitrogen (TN) contents were measured using the Kjeldahl method. For NPN analysis, using Luan et al. method (Luan, Feng, & Sun, 2021) to extract proteins. Then, 10 g of the minced sausage was added with 25 mL of 10% TCA, homogenised, and centrifuged at 4000×*g* at 4 °C for 20 min. Collecting the centrifugation, then

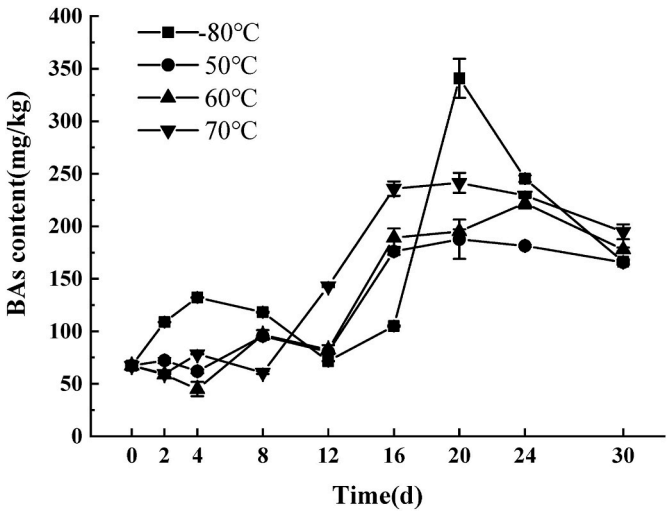


Fig. 6. Effect of oxidised pig backfat on total biogenic amine content in sausage.

determined by the Kjeldahl method.

2.4.2. Determination of protein degradation index (PI)

The proteolysis index was expressed as the percentage ratio between non-protein and total nitrogen (% NPN/TN).

2.4.3. Amino acid nitrogen (AAN)

AAN was determined through the formaldehyde titration method (AOAC, 1990). First, 5.0 g of minced sample was added water to 100 mL, then centrifuged at 5000×*g* for 10 min, and the supernatant was collected. The supernatant of 20.00 mL was added with 60 mL water, and the magnetic stirrer was started. The standard solution of 0.05 mol/L NaOH was titrated to pH 8.2, and 10.00 mL formaldehyde solution was added. Then the standard solution of 0.05 mol/L NaOH was titrated to pH 9.2, and the volume of the standard consumption solution (V1) was recorded. 80 mL distilled water was used to do a blank reagent test under the same conditions, and the volume of the standard consumption solution (V0) was recorded.

2.4.4. Determination of free amino acids

According to the method of Aro et al. (Aro Aro et al., 2010), analysed free amino acids with a slight modification. Briefly, 10 g sausage was cut and mixed with 50 mL of 10% sulfosalicylic acid and precipitated at 4 °C for 2 h. Then, the supernatant was collected after centrifugation and filtration and concentrated to 10 mL using a rotary evaporator (RE-52AA, Shanghai Arong Biochemical Instrument Co., LTD, China) with 0.02 mol/L HCl to 10 mL. Finally, the free amino acids were determined by Automatic Amino Acid Analyser (HITACHI-8900, Hitachi High-tech Corporation, Japan) after the sample was filtered with 0.22 μm membrane.

2.4.5. SDS-PAGE

According to Marino et al.'s method (Marino et al., 2013), albeit with a slight modification, 4 g of the samples was homogenised with phosphoric acid buffer solution A (0.03 mol/L, pH 6.5, W/V = 1:8) and centrifuged at 12,000×*g* at 4 °C for 20 min. The supernatant was filtered as the myofibrillar protein solution. The precipitation was repeated twice, and phosphate buffer B (0.45 mol/L KCl, 15.6 mmol/L Na_2HPO_4, 3.5 mmol/L KH_2PO_4, pH 7.5) was added, followed by centrifugation at 1,2000×*g* at 4 °C for 20 min. The supernatant was filtered as the myofibrillar protein solution. The protein concentration was diluted to 1 mg/mL. A 10 μL sample volume was subjected to 90 V for 30 min on a 12% separation gel and 5% concentration gel and then screened for protein band using Image Lab software.

2.5. Determination of BAs

As suggested by Sun et al. (Sun et al., 2016), 5 g of the meat samples were homogenised with 20 mL of 0.04 mol/L perchloric acid and centrifuged at 4 °C, filtered, precipitated, and extracted as mentioned earlier. The supernatant was collected twice, and the volume of perchloric acid was set to 50 mL. Then, 1 mL of the above extract was collected and added to 200 μL of 2 mol/L NaOH solution, 300 μL of saturated sodium bicarbonate solution, and 2 mL of 10 mg/mL tansyl chloride acetone solution was added in turn. After mixing vigorously, 100 μL of ammonia was added to remove the residual tansyl chloride after a 40-min reaction in the dark at 40 °C. Finally, acetonitrile was added to achieve a volume of 5 mL, and a 0.22 μm filter membrane was used for the liquid phase analysis. Next, the derivative of the BA standard was prepared.

Chromatographic conditions: A C18 column (4.6 × 250, 5 μm) (Agilent Technologies, Palo Alto, CA, USA) was used at the following conditions: flow rate, 0.8 mL/min; UV detector wavelength, 254 nm; injection volume, 10 μL; column temperature, 30 °C; mobile phase A, ultrapure water; mobile phase B, chromatographic grade acetonitrile; gradient elution program was used for separation as follows: 0 min, 35% A + 65% B; 5 min, 30% A + 70% B; 20 min, 0% A + 100% B; 25 min, 35% A + 65% B, for 30 min.

2.6. Statistical analysis

Data were calculated on a dry matter basis to reduce the error between each sampling point. All statistical analyses were performed using Excel, SPSS23.0 software combined with analysis and variance, Duncan's multiple comparison method and Pearson correlation analysis. $P < 0.05$ indicated a significant difference, while $P < 0.01$ indicated an extremely significant difference. We used Origin2019b for drawing. The results of the entire experiment considered the average values of 3 repetitions.

3. Results and discussion

3.1. The POV and TBARS levels of pig backfat under different temperature treatments

POV can reflect the peroxide content of the main primary oxidation products in the lipid oxidation reaction. At the same time, TBARs can mirror the content of secondary oxidation products aldehyde (MDA), as can be seen from Fig. 1(A) that the POV and TBARs of pig backfat increased with increasing temperature. No significant difference was noted in the POV and TBARs between the test groups at −80 °C and −20 °C ($P > 0.05$). With increasing heating temperature, the POV and TBARs of pig backfat increased significantly ($P < 0.05$), which indicated that temperature could substantially affect the oxidation process of lipids. Increasing the heating temperature could significantly promote the formation of lipid peroxides and aldehydes ($P < 0.05$), which was consistent with the results of Li et al. (Li et al., 2019). However, Li et al. didn't consider the effects of microorganisms.

The growth and reproduction of microorganisms could promote lipid degradation and oxidation and deteriorate quality of meat and meat products (Zhang et al., 2021). As evident from Fig. 1(B), at high-temperature heating treatment, the microorganisms were not detected in the pig backfat at 50 °C, 60 °C, 70 °C, and 80 °C. A variety of microorganisms grew in the 2 low-temperature control groups, indicating that high-temperature treatment could effectively kill various microorganisms in pig backfat because the growth and reproduction of these microbes have their corresponding optimum growth temperature. In a specific range, the increasing temperature can promote the growth and reproduction of microorganisms. At extremely high temperatures, the growth of the microorganism is inhibited, and they are killed. Low temperature cannot effectively kill microorganisms; it inhibits the

Table 2
Changes of biogenic amines in Sichuan-style sausage during processing(mg/kg).

		1	2	3	4	5	6	7	8	9
Histamine	−80 °C	NA	2.22 ± 0.14^{abA}	4.99 ± 0.14^{bcA}	6.03 ± 0.34^{cC}	12.95 ± 0.29^{dB}	14.31 ± 0.9^{dA}	44.69 ± 3.37^{fB}	13.93 ± 0.65^{dB}	26.72 ± 2.35^{eBC}
	50 °C	NA	NA	NA	2.11 ± 0.07^{aA}	2.92 ± 0.08^{aA}	21.65 ± 1.26^{dB}	18.79 ± 14^{cA}	10.8 ± 0.59^{bAB}	24.53 ± 1.75^{eB}
	60 °C	NA	NA	NA	3.38 ± 0.45^{aB}	5.01 ± 0.66^{aA}	21.41 ± 1.65^{bB}	20.11 ± 2.43^{bA}	28.06 ± 2.52^{cC}	30.4 ± 1.18^{dC}
	70 °C	NA	NA	NA	3.17 ± 0.51^{aB}	13.35 ± 2.77^{cB}	25.82 ± 0.82^{dC}	34.97 ± 6.79^{eC}	9.11 ± 0.83^{bA}	9.87 ± 0.15^{bcA}
Tyramine	−80 °C	NA	16.48 ± 1.02^{bB}	27.27 ± 0.17^{cC}	36.62 ± 1.31^{dD}	10.78 ± 0.2^{aA}	26.99 ± 0.26^{cA}	70.68 ± 4.53^{fC}	81.93 ± 0.14^{gB}	49.72 ± 0.05^{eA}
	50 °C	NA	10.08 ± 0.04^{aA}	12.57 ± 0.08^{aAB}	20.34 ± 0.12^{bB}	24.98 ± 0.03^{cC}	45.9 ± 0.06^{dB}	54.56 ± 0.18^{fA}	66.42 ± 1.92^{gA}	50.7 ± 2.75^{eA}
	60 °C	NA	10.09 ± 0.09^{aA}	11.28 ± 1.37^{aA}	23.92 ± 1.74^{bC}	19.38 ± 0.13^{bB}	61.4 ± 3.7^{dD}	53.58 ± 0.25^{cA}	64.01 ± 5.37^{dA}	52.13 ± 1.86^{cA}
	70 °C	NA	9.52 ± 0.04^{aA}	13.35 ± 0.08^{aB}	10.79 ± 0.04^{aA}	43.36 ± 0.53^{bD}	59.07 ± 8.55^{cC}	61.18 ± 1.12^{cB}	73.84 ± 5.18^{dAB}	63.03 ± 1.61^{cB}
Cadaverine	−80 °C	19.67 ± 0.97^{bcdA}	18.61 ± 0.07^{bcB}	21.48 ± 1.02^{cdD}	14.55±2^{bA}	6.83 ± 0.29^{aA}	25.23 ± 0.28^{dA}	74.29 ± 7.63^{eB}	69.62 ± 1.63^{eB}	21.79 ± 2.35^{cdA}
	50 °C	19.67 ± 0.97^{bA}	9.74 ± 2.11^{aA}	7.78 ± 1.32^{aB}	10.71 ± 0.06^{aA}	11.46 ± 0.31^{aA}	38.64 ± 0.41^{dB}	30.83 ± 5.31^{cA}	34.79 ± 3.56^{dA}	24.64 ± 0.63^{bA}
	60 °C	19.67 ± 0.97^{cA}	10.8 ± 1.24^{bA}	5.26 ± 0.31^{aA}	11.91 ± 0.11^{bA}	12.93 ± 0.74^{bAB}	34.24 ± 1.05^{eB}	33.23 ± 4.04^{dA}	42.26 ± 3.38^{fA}	28.24 ± 4.19^{dA}
	70 °C	19.67 ± 0.97^{bA}	11.04 ± 0.06^{aA}	11.36 ± 0.43^{aC}	11.15 ± 2.37^{aA}	17.29 ± 2.86^{abB}	44.85 ± 3.81^{dC}	38.88 ± 6.08^{dA}	55.6 ± 1.03^{eB}	30.31 ± 3.72^{cA}
Putrescine	−80 °C	22.91 ± 3.03^{bcA}	24.99 ± 1.12^{cdC}	26.54 ± 1.3^{dB}	20.45 ± 0.34^{bC}	16.01 ± 0.15^{aAB}	24.48 ± 0.69^{cdA}	63.14±0^{gD}	37.04 ± 1.49^{fB}	31 ± 0.43^{eB}
	50 °C	22.91 ± 3.03^{cdA}	10.61 ± 0.49^{aA}	14.78 ± 0.57^{bA}	19.59 ± 0.1^{cC}	13.51 ± 0.3^{abA}	28.69 ± 0.79^{eB}	33.44 ± 2.96^{fA}	28.24 ± 2.41^{eA}	25.72 ± 1.04^{deAB}
	60 °C	22.91 ± 3.03^{cA}	17.63 ± 3.34^{abB}	12.88 ± 2.68^{aA}	15.75 ± 2.72^{aAB}	18.45 ± 1.37^{abcB}	29.59 ± 0.62^{dB}	39.5 ± 2.59^{eB}	35.32 ± 1.96^{eB}	23.82 ± 0.96^{cA}
	70 °C	22.91 ± 3.03^{bA}	15.86 ± 0.28^{aB}	15.24 ± 0.58^{aA}	13.3 ± 1.02^{aA}	24.97 ± 1.96^{bC}	39.87 ± 1.26^{cC}	53.15 ± 1.77^{dC}	35.63 ± 2.67^{cB}	26.9 ± 4.66^{bAB}
Tryptamine	−80 °C	NA	NA	NA	NA	NA	NA	48.69 ± 4.99^{cB}	24.61 ± 1.24^{aA}	38.34 ± 0.7^{bB}
	50 °C	NA	NA	NA	NA	4.87 ± 1.58^{a}	20.57 ± 0.77^{bA}	35.62 ± 3.82^{cC}	20.96 ± 0.82^{bA}	48.72 ± 1.87^{dC}
	60 °C	NA	NA	NA	NA	NA	31.99 ± 2.19^{bB}	15.02 ± 3.93^{aA}	35.43 ± 1.46^{cB}	59.18 ± 0.73^{dD}
	70 °C	NA	NA	NA	NA	NA	47.66 ± 1.05^{cC}	15.77 ± 0.34^{aA}	22.78 ± 1.69^{bA}	22.8 ± 0.05^{bA}
Spermidine	−80 °C	24.65 ± 0.12^{aA}	46.44±0^{dD}	41.89 ± 0.2^{cdD}	40.53 ± 3.51^{bcdB}	23.04 ± 0.99^{aA}	33.5 ± 1.55^{bA}	79.6 ± 0.81^{eB}	47.83 ± 6.2^{dA}	36.98 ± 1.9^{bcA}
	50 °C	24.65 ± 0.12^{aA}	41.77 ± 0.06^{cdC}	26.83±0^{bB}	42.79 ± 0.43^{dB}	27.6 ± 0.1^{bB}	41.12 ± 0.63^{cdAB}	50.01 ± 2.19^{eA}	41.08±1^{cdA}	40.1 ± 0.8^{cA}
	60 °C	24.65 ± 0.12^{abA}	20.27 ± 0.07^{abA}	15.58 ± 2.51^{aA}	41.58 ± 0.5^{cB}	26.72 ± 1.28^{bB}	50.51 ± 8.44^{cdB}	48.68 ± 2.4^{cdA}	52.37 ± 7.66^{dA}	43.31 ± 2.04^{cdA}
	70 °C	24.65 ± 0.12^{aA}	23.17 ± 0.03^{aB}	38.38 ± 0.17^{bC}	22.28 ± 2.58^{aA}	44.02 ± 1.64^{bC}	66.27 ± 16.56^{dC}	53.19 ± 7.35^{cA}	55.05 ± 1.53^{cA}	64.6 ± 6.29^{dB}

The mean value of some biogenic amines between groups on a certain day was expressed by different capital letters (A – D), and the difference was significant ($P < 0.05$). The mean values of some biogenic amines at each sampling point in a group were significantly different with different lowercase letters (a – e) ($P < 0.05$).

Table 3
Correlation analysis among indicators.

Index	POV	TBARs	TN	NPN	PI	AAN	Tryptamine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Total BA
POV	1												
TBARs	.777**	1											
TN	-.354*	-.001	1										
NPN	.158	.592**	.443**	1									
PI	.311	.649**	.115	.939**	1								
AAN	.346*	.431**	-.377*	.444**	.625**	1							
Tryptamine	.200	.453**	.229	.659**	.628**	.402*	1						
Putrescine	.201	.378*	-.186	.423*	.545**	.630**	.569**	1					
Cadaverine	.170	.430**	-.116	.598**	.714**	.693**	.662**	.854**	1				
Histamine	.305	.488**	.025	.513**	.553**	.533**	.771**	.817**	.722**	1			
Tyramine	.456**	.665**	.073	.770**	.822**	.643**	.751**	.720**	.820**	.710**	1		
Spermidine	.367*	.491**	-.077	.467**	.555**	.507**	.636**	.768**	.760**	.717**	.802**	1	
Total BA	.353*	.522**	-.041	.650**	.737**	.673**	.764**	.898**	.922**	.862**	.927**	.893**	1

activity of microorganisms and enzymes to slow the overall growth and reproduction rates.

As can be seen from the results, a high-temperature heating treatment could significantly promote the oxidation process of pig backfat ($P < 0.05$), which was applied for subsequent experiments. As there was no significant difference between the −20 °C and −80 °C

groups, the −80 °C group was selected as the control group.

3.2. Lipid oxidation during Sichuan-style sausage processing

The change of POV in sausage is shown in Fig. 2(A). During the entire fermentation and maturation period, the POV of the 4 treatment groups of sausages showed a trend of first rising and then declining, consistent with the results of Zhao et al. (Zhao, Zhou, Zhang, Pan, & Chen, 2020). Zhao et al. found that POV increased first and then decreased in Chinese dry sausage with different sodium chloride ratios. With an increase in the fermentation and maturation time, the POV of the 4 groups of sausages showed significant differences ($P < 0.05$): the size was in the following order, 70 °C group >60 °C group >50 °C group > −80 °C group. These results indicate that oxidised pig backfat can promote sausage lipid oxidation reaction during fermentation maturation. As shown in Fig. 2(B), during the entire fermentation and maturation period, the TBARs of the 4 groups of sausages showed a trend of first rising and then declining, consistent with the results of Bian et al. (Bian et al., 2019). Bian et al. found that TBARs also increased first and then decreased during the processing of Chinese sausages and were positively correlated with hydroxymethyl linoleic acid. Sausages in the 60 °C group and 70 °C group shared similar TBARs, which were significantly higher than those in the 50 °C and −80 °C group ($P < 0.05$), indicating that oxidised pig backfat can promote the oxidation process and accumulation of lipids during the fermentation and maturation of sausages. More secondary oxidation produces aldehyde compounds.

3.3. Protein degradation during Sichuan-style sausage processing

3.3.1. TN

As shown in Fig. 3(A), during the entire processing period, the TN content of the 4 groups of sausages showed a fluctuating trend, consistent with the research results of Candogan et al. in fermented beef sausage (Candogan, Wardlaw, & Acton, 2009). The decrease in the TN content in the sausages may be attributed to the loss of some water-soluble nitrogenous substances caused by water loss. The increased TN content in sausage may be due to water loss (Yu, Feng, & Sun, 2021). From day 12, TN content in the sausages in groups 50 °C, 60 °C, and 70 °C showed a significant difference (60 °C groups >70 °C group). In other words, the TN content in the sausages decreased with an increase in the oxidation degree of pig backfat during the mid and late mature period. It indicated that oxidised pig backfat might promote protein degradation in the sausages and increase the loss of nitrogen compounds, thereby reducing the TN content in the sausages.

3.3.2. NPN

As shown in Fig. 3 (B), the decrease in the NPN content in sausages may have been due to the loss of some water-soluble nitrogen compounds along with water. The increase in the NPN content indicated that the sausage degraded continually during the maturation period and accumulated FAA and other degradation products (Zhu et al., 2020). After 16 days, the changes in the NPN content in the four groups were irregular. For instance, the contents of NPN in the −80 °C, 50 °C, and 60 °C groups were similar. In contrast, the NPN content in the 70 °C groups was higher than that in the other groups, indicating that treatment of backfat at 70 °C promoted NPN formation in the early and middle stages of sausage maturation.

3.3.3. PI

As shown in Fig. 4 (A), during the entire processing period, the changing trend of PI in the 4 groups of sausages was similar to that of NPN content, indicating a trend of first decreasing and then increasing, suggesting that the degree of protein degradation in sausages increased gradually with time. Before day 24, the changes in PI among the sausages in the experimental group showed apparent regularity and an upward trend, in the following order 70 °C group >60 °C group >50 °C groups, suggesting that the PI of sausages increased with an increase of lipid oxidation degree of pig backfat. This result indicates that oxidised pig backfat could promote the degradation of protein in sausages during fermentation maturity. The changes of PI in the −80 °C group were not consistent with those in the other 3 experimental groups, which may be attributed to the microbial flora in the backfat of the −80 °C group, which increased the degree of protein degradation in the sausage.

3.3.4. AAN

As shown in Fig. 4 (B), during sausage processing, the AAN content of the 4 groups of sausages first increased and then decreased, consistent with the research reports of Latorre-Moratalla et al. (Latorre Moratalla, Bover Cid, Bosch Fusté, & Vidal Carou, 2012). Latorre-Moratalla et al. produced fermented sausage under two different processing conditions, and the AAN of the two groups of sausages increased first and then decreased. IN this study, in the pre-mature and mid-mature stages, the AAN content in the sausages of the experimental groups at 50 °C, 60 °C, and 70 °C exhibited significant differences (60 °C > 50 °C). In other words, the AAN content in the sausages increased with an increase of the oxidation degree of pig backfat, suggesting that the oxidation of pig backfat could promote the degradation reaction of protein and accumulate more FAA in sausages at the pre-mature and mid-mature stages. During this period, the AAN content of sausages in the −80 °C control group was significantly higher than that in the 50 °C groups, which may be attributed to the presence of microorganisms in the pig backfat at −80 °C, which promoted the protein degradation of sausage.

3.3.5. FAA

The FAA content in the 4 groups of sausages on day 30 is shown in Table 1. Large amounts of FAAs were formed after protein degradation in the sausage. FAA is a vital precursor substance for BA formation (Henríquez Aedo, Galarce Bustos, Aqueveque, García, & Aranda, 2018; Kongkiattikajorn, 2015). The highest content of glutamic acid, alanine, and leucine in the 4 sausage groups was inconsistent with Casaburi et al. (Casaburi et al., 2007), which may be attributed to the differences in temperature and time during ripening, resulting in different microbial and protease activities (Domínguez, Munekata, Agregán, & Lorenzo, 2016). The total amount of FAA in the −80 °C, 50 °C, 60 °C, and 70 °C groups were 1.49, 2.09, 2.25, and 2.88 g/100 g, respectively, suggesting that the total amount of FAA in the sausages increased with an increase in the lipid oxidation levels in pig backfat on day 30. Among them, the amino acids related to BA formation mainly included glutamic acid, tyrosine, lysine, histidine, and arginine. Except for arginine, the other 5 amino acids increase with an increase in the lipid oxidation level in pig backfat.

3.3.6. SDS-PAGE

As shown in Fig. 5 (A), in the entire processing, 4 groups of sausage sarcoplasmic protein molecular weight were determined, mainly falling in the range of 25–100 kDa. We discovered that the protein band with a molecular weight of approximately 100 kDa (creatine kinase) disappeared from day 2, and the protein bands with a molecular weight of approximately 25 kDa and 35 kDa gradually deepened, which may be because of the degradation of considerable molecular weight sarcoplasmic proteins into small molecular peptides by endogenous enzymes. In comparison with the 50 °C and 60 °C groups of sausages, in the 70 °C and −80 °C group, the molecular weight of approximately 48 kDa, 35 kDa, and >25 kDa protein band colours at the beginning of day 2 were significantly deepened, which were identified as phosphoglycerate kinase, phosphoglycerate mutase, and pyrophosphate isomerase, respectively (Sun, Cui, Zhao, & Yang, 2011; Visessanguan, Benjakul, Riebroy, & Thepkasikul, 2004). This finding indicated that pig backfat treatment at 70 °C could significantly promote the degradation of the intestinal sarcoplasmic protein.

As shown in Fig. 5 (B), during the entire process, the molecular weights of myofibrillar proteins in the 4 groups of sausages were mainly

25–100 kDa. With increasing time, the colour of the protein band of about 135 kDa molecular weight gradually lightened in colour. The colour of protein bands with molecular weights of approximately 75, 63, 48, and 35 kDa gradually deepened in colour, indicating that myofibrillar proteins in the sausages continually degraded with increasing fermentation maturation time, which is consistent with previous research reports (Chen et al., 2021; Ruiz, Mariscal, & Soriano, 2007). In the middle and late stages of fermentation, the 35-kDa protein bands colour in the 70 °C, 60 °C, and 50 °C groups was significantly deepened on day 16. In contrast, the 70 °C groups colour was greater than that of the 60 °C groups, and the colour of the −80 °C group was significantly deepened on day 20. This finding indicated that oxidised pig backfat could substantially promote the protein degradation process of sausage during fermentation.

3.4. Effect of oxidised pig backfat on BA formation in Sichuan-style sausage

The changes in the total BA content during fermentation of Sichuan-style sausage are shown in Fig. 6. With increasing processing time, the total amount of BA in the sausage showed a trend of first increase and then decreased, as reported by Sun et al. (Sun et al., 2016). The degradation of the generated BA can explain this result by BA oxidase at the late mature stage (Shiling et al., 2010). At the early stage of processing, the total amount of BA in the sausages of 50 °C, 60 °C, and 70 °C groups was similar and maintained stable. From day 12, the total amount of BA in the 50 °C, 60 °C, and 70 °C groups showed a certain regularity, with an increase first and then a decrease, in the following order, 70 °C > 60 °C > 50 °C groups ($P < 0.05$). This result indicated that oxidization of the pig backfat promotes the formation and accumulation of BA in the sausage during the middle and late stages of fermentation.

The changes in the contents of histamine, tyramine, cadaverine, putrescine, tryptamine, and spermidine are shown in Table 2. The four BAs of histamine, tyramine, cadaverine, and putrescine showed nearly the same changing trends as for total amine biogenic during sausage processing. All of these revealed an increase first, followed by a decrease, and the changing trends were in the following order 70 °C > 60 °C > 50 °C groups. This finding suggested that oxidised pig backfat could promote the accumulation of histamine, tyramine, cadaverine, and putrescine in the mid-stage of Sichuan sausage. There was no regular change in tryptamine during the processing of Sichuan sausage, which indicated no apparent regularity between the accumulation of tryptamine and the lipid oxidation degree of raw pig backfat. Spermidine demonstrated a fluctuating change during the entire processing. The changing trend of oxidised pig backfat in the 70 °C groups was significantly greater than in the other experimental groups. At the same time, no significant difference was noted between the 50 °C and 60 °C groups ($P > 0.05$). The −80 °C group showed different changes from the other 3 groups, and there was no apparent regularity, which microorganisms may cause in the pig backfat at −80 °C.

3.5. Correlation analysis

During the entire processing, the correlation among lipid oxidation, protein degradation, and BA formation in the 4 groups of sausages was shown in Table 3. It can be seen from the table that lipid oxidation and protein degradation were closely related in Sichuan-style sausage, and lipid oxidation may promote protein degradation. A significant correlation was noted between lipid oxidation and BA formation ($P < 0.05$), and lipid oxidation may encourage the formation of BA. A significant correlation between protein degradation and BA formation ($P < 0.05$) was noted. Hence, protein degradation may promote the formation of BA.

A large number of studies have shown that lipid oxidation can induce protein oxidation (NimaHematyar, Rustad, & Sampels, 2019; Wazir, Chay, Zarei, & Saari, 2019), and protein oxidation can increase the hydrophobicity of protein surface and change the secondary structure, thus accelerating protein degradation (Zhang, Huang, & Xie, 2019). Therefore, in the current study, we proposed that the incidence of protein degradation in Sichuan sausage may be attributed to protein oxidation inducing by the lipid oxidation.

BAs are formed by free amino acids entering the cells through the cell membrane, decarboxylating in the cells, and transporting them to the outside cells through the cell membrane again (Wang et al., 2021). The phospholipid bilayer is the basic structural element of the cell membrane, and its organization makes a small amount of lipid oxidation affect cell viability, resulting in loss of intracellular balance and increase of permeability. Therefore, lipid oxidation on biogenic amines may be affected by membrane permeability (Runas & Malmstadt, 2014). The rise of permeability makes free amino acids easier to enter the cell, BAs easier to transport out of the cell, and increases biogenic amines' content.

Protein degradation often decomposes into small molecular substances such as amino acids and peptides, which provides a good precursor for the formation of biogenic amines in Sichuan sausage(Zhou et al., 2019). This may be one of the reasons for the significant positive correlation between protein degradation and BAs content.

4. Conclusions

In this study, lipids with oxidation differences obtained at different temperatures were used to produce Sichuan-style sausages. During processing, the contents of POV, TBARs, PI, AAN, FAA, and BA in the sausages of the experimental groups at 50 °C, 60 °C, and 70 °C increased with an increase in the oxidation degree of pig backfat. In the protein electrophoresis, the higher the oxidation degree of pig backfat, the greater was the degradation degree of sarcoplasmic protein and myofibrillar protein in the sausage group. Pearson correlation analyses revealed that lipid oxidation, protein degradation, and BA content correlated significantly ($P < 0.05$).

CRediT authorship contribution statement

Yuxuan Liu: Conceptualization, Methodology, Software, Data curation, Writing – original draft, preparation, Visualization, Investigation, Writing – review & editing. **Yifang Yang:** Conceptualization, Methodology, Software. **Binbin Li:** Conceptualization, Methodology, Software. **Qinjie Lan:** Conceptualization, Methodology, Software. **Xixian Zhao:** Data curation, Writing – original draft. **Yilun Wang:** Data curation, Writing – original draft, preparation, Software, Validation. **Huijie Pei:** Software, Validation. **Xiaohong Huang:** Visualization, Investigation. **Lin Deng:** Visualization, Investigation. **Jianlong Li:** Supervision. **Qin Li:** Supervision. **Shujuan Chen:** Supervision. **Li He:** Supervision. **Aiping Liu:** Supervision. **Xiaolin Ao:** Supervision. **Shuliang Liu:** Supervision. **Likou Zou:** Supervision. **Yong Yang:** Supervision.

Declaration of competing interest

We declared that we have no conflicts of interest to this work.

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