

Effects of Different Moisture Content on the Quality Characteristics of Sichuan Sausage during Frozen Storage

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Abstract Sichuan sausages with moisture contents of 40%, 45%, 50%, and 60% were stored at $-18\text{ }^{\circ}\text{C}$ for durations of 0, 2, 4, 6, and 8 weeks to evaluate the effect of moisture content on the quality attributes of Sichuan sausages during frozen storage. Product indicators including pH, colour, thiobarbituric acid reactive substances (TBARS), total volatile basic nitrogen (TVB-N), texture, electronic nose (E-nose) response, and water-holding capacity (thawing and cooking losses) were determined. The results indicated that as storage time increased, water retention in Sichuan sausages with different moisture contents decreased, while the degree of protein and lipid oxidation increased. This led to an increase in pH value, a colour shift from red-bright to grey-brown, and a deterioration in palatability. Among the samples, sausages with 50% moisture content exhibited the lowest thawing and cooking losses, indicating superior water-holding capacity. After 8 weeks of storage, TBARS and TVB-N values for the 50% moisture group were 19.5% and 2.5% lower, respectively, than those of the 40% and 45% moisture groups, indicating a reduced degree of oxidation. Furthermore, Sichuan sausage with 50% moisture content demonstrated an appropriate pH and colour difference, along with excellent texture and flavour, as evidenced by its higher toughness and satisfactory hardness. In conclusion, Sichuan sausage with 50% moisture content demonstrated the highest overall quality under frozen storage conditions.

Key words Sichuan sausage; Moisture content; Storage time; Quality characteristics

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Sausages represent a quintessential example of traditional Chinese meat products, favoured for their extensive history, unique flavor, and practicality in both storage and processing. Traditionally, sausages are made from minced pork, beef, or mutton^[1], mixed with seasonings, and stuffed into natural casings before being dried, fermented, baked, or air-dried, with or without smoking^[2]. Sausages are primarily categorised into two distinct types: Sichuan and Guangdong flavours^[3]. Among these, Sichuan sausage is especially popular for its characteristic spicy and numbing taste, derived from ingredients such as chilli pepper, Sichuan peppercorn, fermented bean paste, and rice wine added during fermentation^[4].

The moisture content of traditional sausage ranges from 25% to 30%^[5], categorising it as a low-moisture food^[6]. While this low moisture content is advantageous for long-term storage, it can adversely affect the taste and texture, resulting in a product that is often perceived as unpalatable and tough. Chen *et al.*^[7] discovered that the moisture content of certain commercially available Sichuan sausage samples was between 40% and 60%, which was higher than that of traditional Sichuan sausage. Increased moisture content can enhance the texture and flavour of the sausage, making it more appealing to consumers. Consequently, sausages with higher moisture content are likely to become a future trend. However, increased moisture also raises the risk of microbial spoilage

and accelerates chemical deterioration, such as protein and lipid oxidation, during storage^[8], negatively affecting colour, texture, pH, and water-holding capacity. Chen *et al.*^[9] identified yeast and mould as the primary microorganisms responsible for the spoilage of low-salt, high-moisture Sichuan bacon. Additionally, Han *et al.*^[10] observed that during ambient storage of low-temperature sausages, microbial counts increased over time despite reduced bacterial diversity, accompanied by declining sensory quality and increased oxidation. Semi-dry sausages, with moisture contents of 45%–50%, are gaining popularity for their softer texture and improved eating quality compared to traditional low-moisture versions. Nevertheless, the quality attributes of Sichuan sausages within the 45%–60% moisture range remain inadequately studied.

In this study, the water-holding capacity (including thawing loss and cooking loss), pH value, colour, TBARS value, TVB-N value, texture, and flavour of Sichuan sausage were measured with varying moisture contents (40%, 45%, 50%, and 60%) over different storage durations (0, 2, 4, 6, and 8 weeks), aiming to clarify how moisture content influences sausage quality during frozen storage. This study provides a theoretical foundation for the development of high-moisture sausages.

Materials and Methods

Materials

Du Chang Large White pigs (approximately 6–8 months old, weighing between 100 and 110 kg), were sourced for pork from the hind legs and back fat sections, purchased from Iron Knight Food Co., Ltd. (Mianyang, China). The sausage special seasoning was provided by Chengdu Taihefang Brewing Co., Ltd. All chemicals and reagents used in the experiment were of analytical grade.

Sausage samples

Preparation and storage of sausage: Fresh pork 10 kg (fat-thin

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ratio of 3 : 7) → selection and cleaning → trimming and cutting (3 cm × 3 cm × 3 cm) → mixing with mixed bacon seasoning curing (4 °C, 24 h) → mixing and crushing by stirrer → filling (pig small intestine casing, diameter 26 mm) → tie holes, pierce ventilation holes → air-drying (temperature 25 °C, relative humidity 55%, wind speed 0.8 – 1.2 m/s) → finished product (air-dried to sausage moisture content 40%, 45%, 50%, 60%) → vacuum packaging → storage (–18 °C).

Sampling: Sichuan sausages (18 – 20 cm) were dried to moisture content of 40%, 45%, 50%, and 60%. Following vacuum packaging, the sausages were stored in a refrigerator at –18 °C for durations of 0, 2, 4, 6, and 8 weeks, after which relevant indicators were detected and analysed.

Thawing loss rate

The thawing loss rate was determined following the methods described by Hong *et al.* [11], with slight modifications. Sichuan sausages stored for 0, 2, 4, 6 and 8 weeks were taken out of the refrigerator at –18 °C and quickly weighed (m_1), and then the sausages were transferred to a refrigerator at 4 °C for 24 h to thaw. After the sausages were completely thawed, the surface moisture of the Sichuan sausage was absorbed using a paper towel, and the mass (m_2) was weighed with an electronic analytical balance. Each group consisted of three parallel samples, and the average mass was calculated. The thawing loss rate of Sichuan sausage was calculated according to Formula (D_{LR}):

$$D_{LR} = \frac{m_1 - m_2}{m_1} \times 100\%$$

Cooking loss rate

After the Sichuan sausages stored at –18 °C for 0, 2, 4, 6, and 8 weeks were completely thawed, the surface moisture was absorbed with a kitchen paper towel, and the mass before cooking (m_3) was weighed. The Sichuan sausages were packed in a vacuum bag and cooked in a thermostatic water bath at 90 °C. Once the internal temperature reached 75 °C, the sausages were promptly removed and cooled to room temperature. The surface exudates and moisture of the sausages were wiped off with a kitchen paper towel, and the mass of the Sichuan sausage after cooking (m_4) was weighed. Each group was tested in triplicate, and the results were averaged. This procedure was based on the method described by Gan *et al.* [12], with slight modifications. The cooking loss rate (C_{LR}) of Sichuan sausage was calculated according to Formula (C_{LR}):

$$C_{LR} = \frac{m_3 - m_4}{m_3} \times 100\%$$

Analysis of chroma value

After the Sichuan sausages with moisture contents of 40%, 45%, 50%, and 60% and storage periods of 0, 2, 4, 6, and 8 weeks had been completely thawed, 5 cm segments of meat were cut. Following peeling off the casing, the meat was placed in a food processor and minced. The resulting mince was then spread into an even flat layer measuring 8 cm in diameter. The L^* , a^* and b^* values were measured using a CR-400 colour difference

meter [13]. Each group of samples was measured six times, and the average value was taken.

pH value

The pH value was determined following the methods described by Liu *et al.* [14]. After Sichuan sausages with moisture contents of 40%, 45%, 50%, and 60% and storage periods of 0, 2, 4, 6, and 8 weeks had been fully thawed, 5 cm segments of meat were cut. The meat-specific puncture electrode of the pen pH meter was inserted into the sausage segment for detection. Each sample group was measured three times, and the average value was recorded.

TBARS value

The TBARS value was determined according the methodology established by Biliska *et al.* [15] with appropriate modifications. A reagent blank was prepared, and the absorbance was recorded at 532 nm. Each sample group was measured three times. The TBARS value was calculated using the standard curve, and the malondialdehyde standard solution was prepared with trichloroacetic acid.

TVB-N value

According to the determination method described by Gu *et al.* [16], the sausage filtrate was evaporated in an alkaline solution and absorbed by boric acid solution. Each group of samples was measured three times to evaluate the freshness of Sichuan sausage with different moisture contents during frozen storage.

Shearing force

The shear force was measured following the method outlined by Cao *et al.* [17] with some modifications. After determining the cooking loss rate, the sausage was cooled to room temperature and cut into a uniform cylinder with a height of 2 cm. A texture analyser was employed to measure the shear force. Measurements were performed with an HDP/BSW probe at a strain height of 10 mm, using test speeds of 2.00 mm/s (pre-test), 1.00 mm/s (test), and 2.00 mm/s (post-test). The target displacement was set to 35.000 mm with an automatic trigger force of 20.0 g.

Texture profile analysis (TPA)

After detecting the cooking loss rate, the sausage was cooled to room temperature and cut into 1 cm-high uniform cylinders. Texture profile analysis (TPA) was conducted using a texture analyser [18], configured with a P/50 probe in TPA mode. The test was performed at a constant speed of 1.00 mm/s throughout all phases (pre-test, test, and post-test), with a target strain of 40% and an automatic trigger force of 3.0 g. A 3.00 s interval was set between the two compressions.

Electronic nose

The sausages of each experimental group were peeled off the casings and crushed. Then, 3.00 g of each sample was accurately weighed into a headspace bottle. Before the determination, the headspace bottle was placed in a 40 °C water bath for equilibrium for 20 min [19]. The flavour of the samples was analyzed using a PEN 3 electronic nose detector. The measurements were performed with following parameters: a sampling interval of 1.0 s, a sensor

cleaning time of 50 s, a pre-injection time of 5 s, and a measurement time of 60 s. The carrier gas flow rate was set to 400 ml/min for both the sensor and the sample. Each group of samples was measured three times.

Statistical analysis

Each experiment was conducted in triplicate, and the data were analysed using IBM SPSS Statistics 29.0 software. The results were expressed as mean \pm standard deviation, and $P < 0.05$ indicated that there was a significant difference between the data. Excel 2016 software was used to process the data, and Origin 2021 and SIMCA version 14.1 were utilised for graphical representation.

Results and Discussion

Effect of moisture content on the water-holding capacity of Sichuan sausage

Thawing loss rate Thawing loss refers to the ability of meat products to retain moisture during the thawing process, serving as an indicator of meat freshness^[20]. The extent of thawing loss reflects the effects on the quality and texture of sausages throughout freezing, frozen storage, and thawing. A high thawing loss rate in Sichuan sausage may result in reduced quality and inferior texture, whereas a low thawing loss rate indicates the sausages' superior preservation properties^[21].

As shown in Fig. 1, different moisture contents exerted varying effects on the thawing loss of Sichuan sausages. Fresh Sichuan sausages (those not subjected to frozen storage) exhibited no thawing loss. As storage duration increased, thawing loss in the sausages demonstrated a decreasing trend. After 2 weeks of storage, the thawing loss for sausages with moisture contents of 40%, 45%, 50%, and 60% was recorded at 0.88%, 1.87%, 1.29%, and 1.57% respectively. After 8 weeks of storage, these values decreased by 0.82%, 0.76%, 0.11%, and 0.02% respectively. Throughout this period, the thawing loss decreased by 0.06%, 1.11%, 1.18%, and 1.55%, respectively, indicating a significant reduction in thawing loss in Sichuan sausages with increasing storage duration ($P < 0.05$). In addition, the thawing loss of Sichuan sausages with 40% and 50% moisture content was significantly lower than that of 45% and 40% sausage for the same storage time. At 6 weeks of storage, the thawing loss for Sichuan sausages with moisture contents of 40% and 50% was 0.95% and 1.61% lower, respectively, than that for sausages with 45% moisture content. The experimental results indicated that moisture content significantly influenced the water-holding capacity of Sichuan sausages. Specifically, sausages air-dried to 40% and 50% moisture content exhibited superior water-holding capacity compared to those with 45% and 60% moisture content. Notably, the thawing loss in the 50% moisture group decreased significantly after 4 weeks of storage, stabilising at 0.11% after 6 and 8 weeks. This phenomenon may be attributed to the formation and growth of ice crystals during freezing and storage, which disrupts the internal structure of the sausages. Higher moisture content leads to the precipitation of more ice crystals, thereby reducing the

water-holding capacity of the sausages. This finding aligns with the results of Zhang *et al.*^[22], which demonstrated that an increase in the moisture content of golden thread fish sausage raises the freezing point and subsequently increases thawing loss. In addition, although all samples were frozen at $-18\text{ }^{\circ}\text{C}$, the thawing loss showed an overall decreasing trend with increasing storage time, increasing ($P < 0.05$) at 6 weeks of storage. This phenomenon may be because with the extension of time, the small ice crystals inside the Sichuan sausage gradually converge to the large ice crystals, and recrystallisation occurs. This process transforms the initially ordered and stable nuclei of the internal ice crystals into chaotic, larger structures, which can puncture muscle tissue and cause mechanical damage^[23]. Consequently, this damage leads to increased water outflow from the membrane, significantly elevating thawing loss.

Steaming loss rate Steaming loss is a critical indicator for evaluating the water-holding capacity of meat products. During thermal processing, the muscle structure degrades due to myofibrillar denaturation, resulting in the exudation of a significant amount of water and soluble substances from the product. This process significantly affects the yield of meat products^[24]. The cooking loss of Sichuan sausages to moisture contents of 40% and 45% exhibited a trend of initially increasing, then decreasing with extended storage duration, peaking at 6 weeks. In contrast, Sichuan sausages with moisture contents of 50% and 60% exhibited a pattern of initial decrease followed by an increase and subsequent decrease. The formation of large, uneven ice crystals during prolonged freezing altered the structure and functional properties of myofibrillar proteins, thereby increasing the cooking loss rate of the sausages^[25]. Conversely, short-term freezing had a lesser impact on the cooking loss of sausages with lower moisture content.

At zero weeks of storage, the cooking loss rate of sausages showed a positive correlation with moisture content. Sausages with higher moisture content exhibited greater evaporation and loss during cooking, leading to increased cooking losses. Sausages with 60% moisture content demonstrated a cooking loss rate that was 3.26% higher than those with 40% moisture content. At 4 weeks of storage, sausages with 50% moisture content exhibited the lowest cooking loss rate. By 6 weeks of storage, cooking loss rates across all groups showed an upward trend, reaching peak values, with sausages at 45% moisture content recording the highest cooking loss rate of 6.02%. As the storage duration increased, the cooking loss rates across all experimental groups initially rose and then declined, consistent with the findings of Suliman *et al.*^[26]. This occurs because during storage, moisture redistributes internally within the sausages while surface moisture evaporates. However, internal moisture stabilises, which reduces water loss during cooking. Concurrently, protein and fat undergo cross-linking or solidification, forming a more stable structure that resists loss during cooking, thereby decreasing the loss rate.

Effect of moisture content on the colour of Sichuan sausage

One of the primary factors influencing consumers' decisions to

purchase cured sausages is colour. The characteristics of lightness, redness, and yellowness in meat products are represented by the measured L^* , a^* , and b^* values, respectively^[27]. The L^* , a^* , and b^* values of Sichuan sausages decrease as moisture content increases, since higher moisture dilutes pigments and fats, resulting in a paler surface colour, reduced gloss, and diminished red and yellow hues. With extended storage duration, the L^* and a^* values of cured sausages decrease. This occurs because reduced moisture content during the air-drying phase diminishes light transmittance through the muscle tissue, darkening the surface^[28]. Concurrently, the oxidation of myoglobin and oxyhaemoglobin into methaemoglobin, coupled with Maillard reactions, causes the redness value to diminish progressively, resulting in a shift towards brown or grey hues. Conversely, the b^* value typically

increases with storage duration. This is generally attributed to yellow spots appearing on the sausage surface due to oxidation of meat products and microbial proliferation^[29], causing elevated b^* values. This observation aligns with findings by Li *et al.*^[30], who observed significant decreases in L^* and a^* values alongside a slight increase in b^* with extended frozen storage. As shown in Table 1, the L^* value of sausages with 60% moisture content was 5 – 10 units lower than that of sausages with 40% moisture content. Moreover, the longer the storage duration, the greater the colour difference between the two groups. The sausages with 50% moisture exhibited moderate colour. After 8 weeks of storage, the b^* value of all four sausage groups averaged 2 units higher than at 0 weeks.

Table 1 Effect of moisture content on the colour difference of Sichuan sausage

Color	Storage period week	Sample			
		40%	45%	50%	60%
L^*	0	50.16 ± 0.50 ^{Aa}	46.34 ± 2.65 ^{Ab}	45.76 ± 0.34 ^{Bb}	37.6 ± 0.57 ^{Cc}
	2	48.2 ± 0.65 ^{Ab}	48.67 ± 0.96 ^{Ab}	46.38 ± 2.08 ^{Ab}	54.1 ± 1.80 ^{Aa}
	4	47.89 ± 1.53 ^{Ba}	47.59 ± 2.59 ^{Aa}	46.13 ± 2.46 ^B	43.15 ± 0.91 ^{Bb}
	6	53.34 ± 0.11 ^{Aa}	46.79 ± 2.04 ^{Ab}	46.68 ± 1.82 ^{Ab}	44.75 ± 0.24 ^{Cb}
	8	43.09 ± 0.76 ^{Da}	42.21 ± 1.89 ^{Ba}	34.78 ± 0.04 ^{Cb}	32.13 ± 1.90 ^{Cc}
a^*	0	15.88 ± 0.84 ^{Aa}	15.69 ± 1.70 ^{Aa}	10.76 ± 1.67 ^{BCb}	10.33 ± 1.65 ^{Ab}
	2	14.55 ± 1.98 ^{Aa}	15.06 ± 1.86 ^{Aa}	11.91 ± 0.74 ^{Aa}	12.39 ± 1.43 ^{BA}
	4	15.88 ± 0.84 ^{Aa}	13.02 ± 1.61 ^{Ab}	13.67 ± 1.31 ^{Aab}	12.57 ± 0.87 ^{Ab}
	6	15.05 ± 1.44 ^{Aa}	13.56 ± 2.14 ^{Aab}	11.95 ± 0.18 ^{Ab}	11.62 ± 0.84 ^{Ab}
	8	11.87 ± 2.13 ^{Aa}	10.51 ± 0.77 ^{Ca}	9.71 ± 2.73 ^{Ba}	9.53 ± 0.90 ^{Ca}
b^*	0	14.05 ± 0.64 ^{Aa}	16.43 ± 2.00 ^{Aa}	14.06 ± 1.69 ^{Ba}	16.56 ± 1.46 ^{Ba}
	2	9.19 ± 1.90 ^{Ba}	11.13 ± 2.06 ^{Ba}	10.85 ± 0.88 ^{Ca}	10.24 ± 0.72 ^{Ca}
	4	15.33 ± 3.10 ^{Aab}	16.82 ± 3.84 ^{Aa}	11.47 ± 0.81 ^{Cb}	12.03 ± 0.96 ^{Cab}
	6	13.22 ± 1.09 ^{Aa}	14.57 ± 1.05 ^{Aab}	14.88 ± 1.35 ^{Aab}	19.57 ± 1.09 ^{Aa}
	8	16.27 ± 1.97 ^{Aa}	17.47 ± 3.03 ^{ABa}	16.78 ± 1.13 ^{ABa}	18.31 ± 0.62 ^{Aab}

Effect of moisture content on the pH value of Sichuan sausage

The pH value significantly influences the overall flavour and quality characteristics of Sichuan sausages, and can be used to measure the freshness of preserved sausages, determine their storage time and quality changes. As illustrated in Fig. 3, the pH values of all four groups of Sichuan sausages were weakly acidic ($4 < \text{pH} < 6$), primarily due to the acid production from lactic acid fermentation during the air-drying and storage processes. With the exception of the two-week storage period, the pH of the preserved sausages increased alongside the moisture content^[31]. The air-drying time for preserved sausages with high moisture content during the initial storage phase was shorter, which led to the restriction of lactic acid bacterial activity and the inability to produce lactic acid efficiently. In the late stage of storage, high moisture content accelerated the oxidation of proteins and fats, leading to the production of alkaline substances (*e.g.*, peptides and amines, *etc.*), and the high moisture content was unable to inhibit the growth and reproduction of microorganisms, which led to an increase in the pH value rises. With the prolongation of storage time, the pH value of Sichuan sausages, except for the group with

50% moisture content, exhibited a general trend of decreasing followed by increasing, which is consistent with the results of Zhou *et al.*^[32]. The pH values at week 0 and week 8 were not significantly different ($P > 0.05$). Specifically, for sausages with 45% moisture, pH was 5.29 initially and 5.24 after 8 weeks. For those with 60% moisture, it was 5.59 and 5.73, respectively. In the early stage of storage, microorganisms such as lactic acid bacteria convert carbohydrates into acidic substances such as lactic acid, resulting in a decrease in the pH of sausage^[33]. When storage time is too long, protein decomposition produces alkaline substances such as amines, resulting in a rise in pH^[34]. The pH of sausages with 50% moisture content increased at week 2 of storage. This temporary rise could be attributed to the weaker ability to inhibit protein decomposition and the production of alkaline substances (*e.g.*, peptides and amines) from protein degradation. In contrast, the pH of sausages with 40% moisture content decreased significantly by week 8 ($P < 0.05$). This is likely because the lower moisture content favored lactic acid bacteria fermentation and led to a higher concentration of carbohydrates, promoting the accumulation of acidic substances such as lactic and

acetic acid.

Effect of moisture content on the TBARS value of Sichuan sausage

More than half of the flavour compounds in meat products are derived from lipid oxidation, a process that generates malondialdehyde, which serves as an indicator of the extent of lipid oxidation^[35]. The TBARS value assesses the degree of lipid oxidation through the detection of malondialdehyde, with higher values indicating a greater degree of lipid oxidation, leading to diminished flavour and quality of the sausage^[36]. As illustrated in Fig. 4, the TBARS values of Sichuan sausages with different moisture contents exhibited an overall increasing trend over the storage period, which is consistent with the results of the study on TBARS values of 3D-printed pork pavement during the storage period conducted by Wang *et al.*^[37]. The preserved sausages with 40% and 60% moisture contents showed the greatest increase, which were increased by 121.16% and 246.20%, respectively, and the difference was significant ($P < 0.05$). The 50% experimental group took the second place, and the smallest increase was in the 45% experimental group. This suggests that higher moisture content promotes fat oxidation and microbial growth, thereby accelerating the oxidative spoilage of sausage. Notably, the increase in TBARS values for preserved sausages was particularly pronounced during the first two weeks of storage compared to later stages. As the fat in preserved sausages continued to oxidise, malondialdehyde gradually increased and was further oxidised to other organic acids and alcohols, or Schiff bases produced by the action of Schiff bases with other amines prevented the binding of aldehydes to thiobarbituric acid^[38], resulting in a decrease in the readily oxidizable components of the preserved sausages. Furthermore, moisture loss in the later stages of storage, due to icing, reduced water activity and the number of water molecules available for oxidation reactions, leading to a decrease in the oxidation degree of Sichuan sausages during this period^[39].

Effect of moisture content on the TVB-N value of Sichuan sausage

TVB-N is an alkaline nitrogen-containing volatile compound with an irritating odour produced by the decomposition of proteins in meat products under the action of enzymes and microorganisms, which can be converted into carcinogenic N-nitroso, and is used to determine the degree of freshness of meat products^[40]. A higher TVB-N value indicates a lower degree of freshness in Sichuan sausages. As shown in Fig. 5, the TVB-N values of sausage increased progressively with storage time, indicating a concomitant rise in protein oxidative decomposition. This finding aligns with Wu's^[41] study, which reported that the TVB-N content of camel meat increased with extended refrigeration time ($P < 0.05$). Specifically, during the 8 weeks of storage, the moisture content of 40%, 45%, 50% and 60% increased their TVB-N values by 234.34%, 261.67%, 226.15% and 167.6%, respectively, compared to their TVB-N values at week 0. The data also indicate a positive correlation between moisture content and TVB-N values.

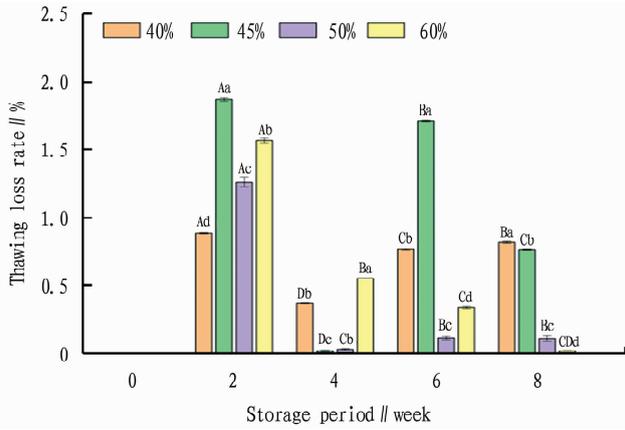
For instance, after six weeks of storage, sausages with 40% moisture content displayed a TVB-N value 12.96 mg/100g lower than those with 60% moisture content. This occurs because lower moisture content reduces the mechanical damage inflicted by ice crystals upon proteins, thereby further slowing protein degradation^[42]. Additionally, a lower moisture environment is unfavourable for the growth and reproduction of microorganisms and enzymes, thus reducing the degree of oxidation of Sichuan sausages.

Effect of moisture content on the shear force of Sichuan sausage

Shear force is an important indicator of the texture and flavour of meat products. For preserved sausages, a higher shear force corresponds to lower tenderness and, consequently, reduced taste^[43]. The effect of different moisture content on the shear force of Sichuan sausage is shown in Fig. 6. With the increase of storage time, the shear force of preserved sausages with 40% and 45% moisture content showed a decreasing and then increasing trend, while the shear force of preserved sausages with 50% and 60% moisture content showed an increasing and then decreasing trend. The initial increase in shear force over time was attributed to the denaturation, degradation, and contraction of myofibrillar proteins, which contributed to the hardening of the sausage^[44], whereas the decrease in shear force was attributed to the oxidative breakdown of fats and proteins to produce small molecules as well as to the breakdown of muscle fibres and connective tissues by endogenous enzymes (*e.g.*, proteases and lipases) or Ca^{2+} , which resulted in the softening of the texture of sausage^[45]. At 2 weeks of storage, there was less ice crystal formation in the sausage with high moisture content than at the later stage of storage, and it lost a significant amount of soluble proteins and water during cooking^[46], which resulted in lower tenderness of sausage with high moisture content than that of sausage with low moisture content. The shear force of preserved sausage in the late storage period was negatively correlated with moisture content, and when stored for 6 weeks, the shear force of preserved sausage with 40% moisture content was higher than that of the preserved sausage in the 60% moisture content group by 9,767.83 N. A lower moisture content led to an increase in the shear force of the preserved sausages. This is attributed to the hardening of myofibril and connective tissue structures under drier conditions. Our finding aligns with the report by Zhang *et al.*^[47], who observed that decreased moisture content resulted in higher hardness and chewiness in pork tenderloin, showing an overall inverse correlation.

Effect of moisture content on the texture and slice morphology of Sichuan sausages

The texture of sausage is one of the main factors affecting consumers' purchasing desire. A texture instrument was employed to simulate the chewing movements of the human oral cavity, allowing for the objective evaluation of sensory attributes of preserved sausage^[48], including hardness, elasticity, and chewiness. Commercially available sausage should have moderate hardness, elasticity and favourable cohesion and chewiness. As shown in Table 2,



Lowercase letters (a-d) denote significant differences between storage periods at the same moisture content, while uppercase letters (A-D) indicate significant differences between storage periods at the same moisture content ($P < 0.05$). The same applies below.

Fig. 1 Effect of moisture content on the thawing loss of Sichuan sausage

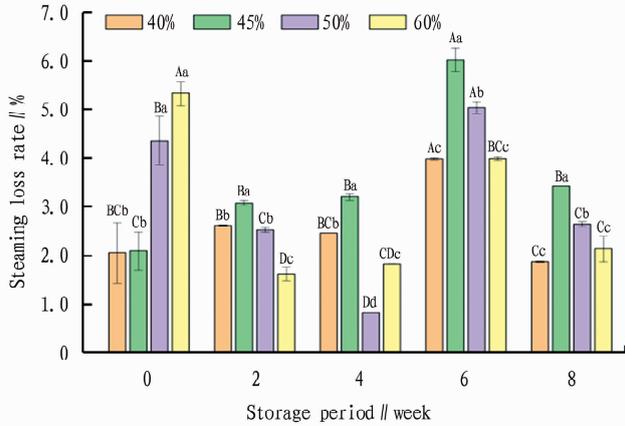


Fig. 2 Effect of moisture content on the cooking loss of Sichuan sausage

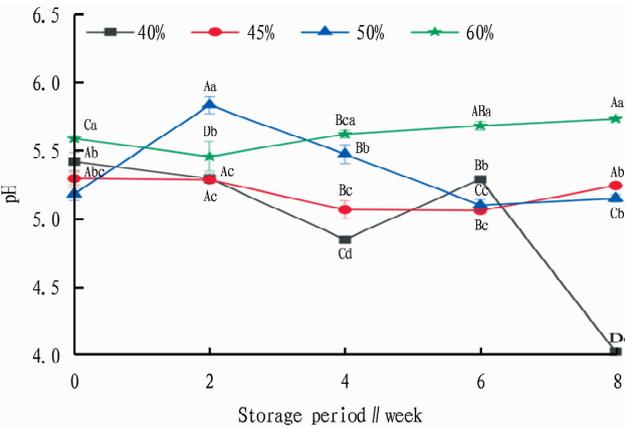


Fig. 3 Effect of different moisture content on the pH value of Sichuan sausage

the observed increase in hardness of preserved sausage over extended storage periods can be attributed to moisture loss^[49] which leads to the formation of ice crystals, and low-temperature storage that accelerates fat crystallisation, which will further increase

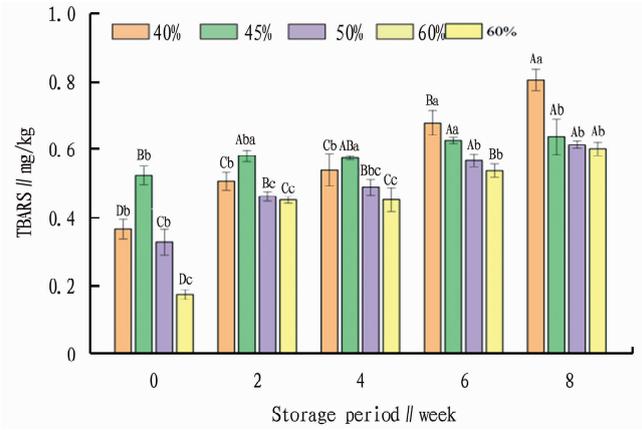


Fig. 4 Effect of moisture content on the TBARS value of Sichuan sausage

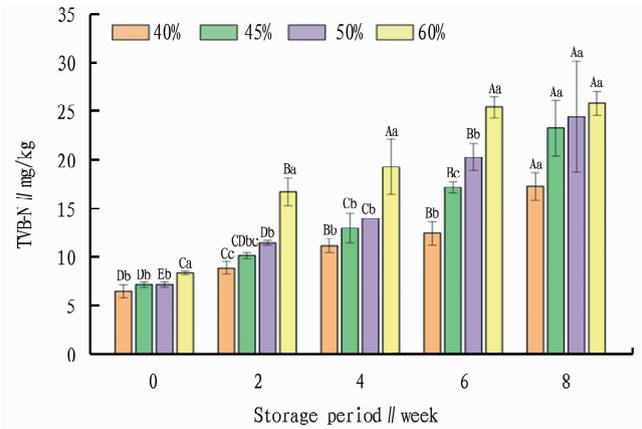


Fig. 5 Effect of different moisture content on TVB-N value of Sichuan sausage

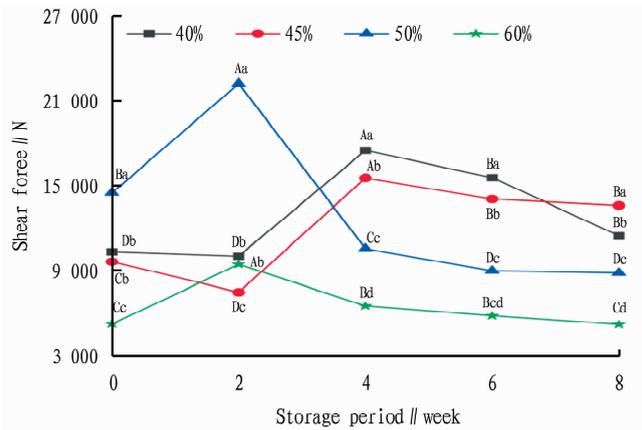


Fig. 6 Effect of different moisture content on the shear force of Sichuan sausage

the hardness of preserved sausage. For instance, preserved sausage with a moisture content of 45% experienced a 39.7% increase in hardness after 8 weeks of storage compared to its initial state. Conversely, the elasticity and cohesiveness of preserved sausage were inversely related to storage time, and the oxidation of proteins in preserved sausage during storage led to changes in its three-dimensional structure, as well as the decomposition of muscle

fibres and connective tissues by endogenous enzymes and the oxidation of fats decreased the elasticity of preserved sausage. This is consistent with the results of Shi *et al.* [50], who reported that the spatial structure of yak meat muscle tissue was compromised by freezing and thawing processes, resulting in a loss of intrinsic elasticity. Additionally, as the moisture content of sausage increases, its hardness decreases [51]. This phenomenon occurs because higher moisture levels promote the dispersion of fat and protein, thereby weakening the interactions between them and resulting in a softer texture. Furthermore, the elasticity and cohesion of sausage are positively correlated with moisture content, as moisture enhances the softness and plasticity of the internal structure and improves the hydration of the protein network. For example, after 8 weeks of storage, sausage with a moisture content of 60% exhibited greater elasticity and cohesion than that with 40% moisture, with differences of 0.293 mm and 0.117, respectively. Consequently, sausage with higher moisture content demonstrated superior elasticity and cohesion [52]. The chewiness of sausage followed

a sausage trend to hardness, increasing with longer storage times and lower moisture content, as greater hardness necessitated more force for chewing.

The sausages were sliced after cooking or sliced and fried for consumption, so the effects of storage time and moisture content on the quality of sausages could be analysed intuitively by observing the slice morphology. As shown in Fig. 7, the redness value of sausage with 60% moisture content was reduced compared with that of sausage with 40% moisture content, and sausage stored for 8 weeks was greyish-brown compared with that at the beginning of the storage period, which was consistent with the results of the colourimeter. Furthermore, as storage time increased, the looseness of the sausage slices also increased, resulting in a wider gap between fat and lean meat, with noticeable cracks appearing in the slices of sausages with 40%, 45%, and 50% moisture content after 8 weeks of storage. The looseness of sausage slices decreased with higher moisture content, corroborating the findings of Sichuan sausage cohesion measured by the texture instrument.

Table 2 Effect of moisture content on the texture of Sichuan sausage

Texture	Storage period//week	Sample			
		40%	45%	50%	60%
Hardness//g	0	17 171.10 ± 3 950.02 ^{Ba}	17 959.76 ± 4 545.86 ^{Ba}	17 233.59 ± 2 571.32 ^{A^{Ba}}	6 417.09 ± 6 101.67 ^{Bb}
	2	22 367.71 ± 1 138.06 ^{ABa}	14 348.03 ± 9 545.40 ^{Bab}	13 947.21 ± 1 532.34 ^{A^{Bab}}	10 061.50 ± 8 902.61 ^{ABb}
	4	22 633.91 ± 11 160.26 ^{ABa}	20 675.17 ± 6 969.89 ^{ABa}	9 693.86 ± 6 826.31 ^{Cb}	10 767.56 ± 1 251.79 ^{ABb}
	6	24 491.42 ± 6 526.39 ^{Aa}	20 723.66 ± 2 752.70 ^{ABa}	10 793.93 ± 7 246.31 ^{Bb}	10 844.10 ± 3 486.33 ^{Bb}
	8	26 075.19 ± 921.17 ^{Aa}	25 087.31 ± 1 413.54 ^{Aa}	18 556.10 ± 596.36 ^{Ab}	11 890.25 ± 1 295.95 ^{Ac}
Springiness//mm	0	0.744 ± 0.17 ^{Aab}	0.825 ± 0.08 ^{Aab}	0.756 ± 0.02 ^{Aab}	0.855 ± 0.05 ^{Aa}
	2	0.728 ± 0.06 ^{Abc}	0.72 ± 0.06 ^{Ab}	0.720 ± 0.08 ^{Ac}	0.827 ± 0.03 ^{Aa}
	4	0.604 ± 0.34 ^{Ab}	0.726 ± 0.03 ^{Aa}	0.688 ± 0.83 ^{Ab}	0.764 ± 0.04 ^{ABa}
	6	0.671 ± 0.02 ^{Aa}	0.783 ± 0.06 ^{Aa}	0.556 ± 0.37 ^{Bb}	0.763 ± 0.07 ^{ABa}
	8	0.433 ± 0.40 ^{Bb}	0.540 ± 0.31 ^{Bb}	0.610 ± 0.08 ^{Ba}	0.726 ± 0.03 ^{ABa}
Cohesiveness	0	0.676 ± 0.05 ^{Aab}	0.657 ± 0.03 ^{Ab}	0.61 ± 0.02 ^{Bb}	0.725 ± 0.02 ^{Aa}
	2	0.515 ± 0.30 ^{Ba}	0.603 ± 0.03 ^{ABa}	0.732 ± 0.17 ^{Aa}	0.664 ± 0.03 ^{ABa}
	4	0.547 ± 0.34 ^{Aa}	0.514 ± 0.10 ^{Ba}	0.515 ± 0.34 ^{ABa}	0.727 ± 0.52 ^{Aa}
	6	0.551 ± 0.02 ^{Aa}	0.590 ± 0.00 ^{ABa}	0.614 ± 0.07 ^{Ba}	0.590 ± 0.08 ^{Ba}
	8	0.531 ± 0.00 ^{Bb}	0.546 ± 0.03 ^{Bb}	0.551 ± 0.07 ^{Bb}	0.648 ± 0.04 ^{ABa}
Gumminess//mJ	0	5 186.06 ± 5 987.87 ^{Ca}	8 553.84 ± 2 492.67 ^{Ba}	4 723.92 ± 4 028.99 ^{Ab}	2 680.28 ± 1 907.66 ^B
	2	8 710.72 ± 2 455.56 ^{Aa}	9 776.31 ± 448.31 ^{Bab}	4 538.54 ± 3 420.09 ^{Aa}	3 814.83 ± 3 520.81 ^{ABa}
	4	7 025.85 ± 4 820.04 ^{Ba}	9 130.94 ± 226.85 ^{Ab}	3 446.28 ± 3 575.93 ^{Ac}	5 963.48 ± 953.23 ^{Aa}
	6	9 041.32 ± 2 384.75 ^{Ab}	9 460.31 ± 267.58 ^{Ba}	7 344.40 ± 620.07 ^{Abc}	6 575.81 ± 736.39 ^{Ac}
	8	13 138.45 ± 1 096.21 ^{Aa}	10 406.77 ± 1 276.06 ^{Ba}	7 925.60 ± 890.27 ^{Aa}	7 074.70 ± 922.83 ^{ABb}

Effect of moisture content on the odour difference of Sichuan sausages

The electronic nose generates signals for the volatile odour in Sichuan sausages by mimicking human olfactory perception through an array of gas-sensitive sensors, and then outputs qualitatively or quantitatively the results of the detection of the components contained in the gas [53]. The effect of different moisture

content and storage time on the volatile flavour of preserved sausages are shown in Fig. 8, where preserved sausages from all experimental groups were more sensitive to W1W (sensitive to hydrogen sulphide), W1S (sensitive to methane), W2W (sensitive to hydrogen sulphide analogues), W2S (sensitive to ethanol), and W5S (sensitive to nitrogen oxides). The highest response values for W1S and W5S were found in sausage stored for 8 weeks, indicating

an increase in nitrogen oxides and methyl analogues during the spoilage process^[54]. The principal component analysis (PCA) of the volatile flavours of Sichuan sausages with different moisture contents and storage time yielded cumulative contributions of 93.2% and 81.5%, respectively. This suggests that the two PCAs effectively represent the primary informative characteristics of preserved sausage flavours. From the PCA plots, it can be seen that the flavour differences between sausages with 50% and 60% moisture content and those with 40% and 45% moisture content were larger, which was related to the effect of moisture on protein structure and lipid oxidation; and the flavour differences between sausages stored for 0 and 2 weeks and those stored for 4, 6 and 8 weeks were larger, which indicated that the flavour substances of Sichuan sausages were changed during the storage period.

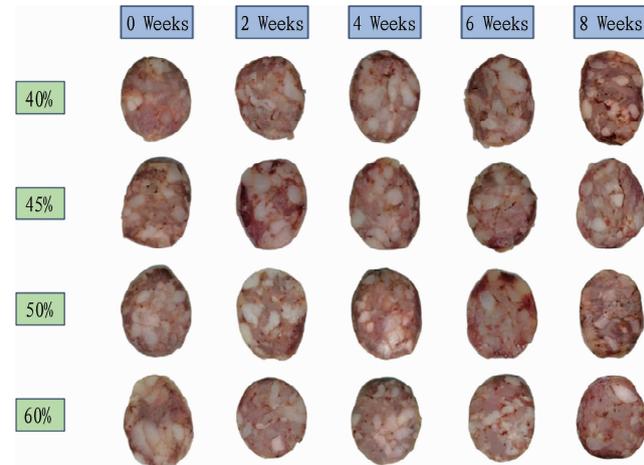


Fig. 7 Effect of different moisture content on the morphology of Sichuan sausage

Conclusions

In this study, we investigated the effects of water-holding capacity (thawing loss, cooking loss), colour difference, pH, TBARS, TVB-N, texture, and odour of preserved sausages with moisture contents of 40%, 45%, 50%, and 60% when stored at -18°C for durations of 0, 2, 4, 6, and 8 weeks. The results indicated that prolonged storage resulted in decreased water retention and increased oxidation of proteins and lipids across all samples, accompanied by elevated pH, a colour shift from reddish to grey-brown, and diminished palatability. Among the four experimental groups with different moisture contents, sausages with 50% moisture content exhibited superior water retention capacity, lower oxidative degradation, and more suitable colour and structural organisation during storage. This study provides theoretical support and a scientific basis for the processing and storage of Sichuan sausage. Future research can further analyse the effects of moisture content and storage time on the quality and flavour of Sichuan sausage through analysis of microbial communities, compositional changes, and flavour compound analysis.

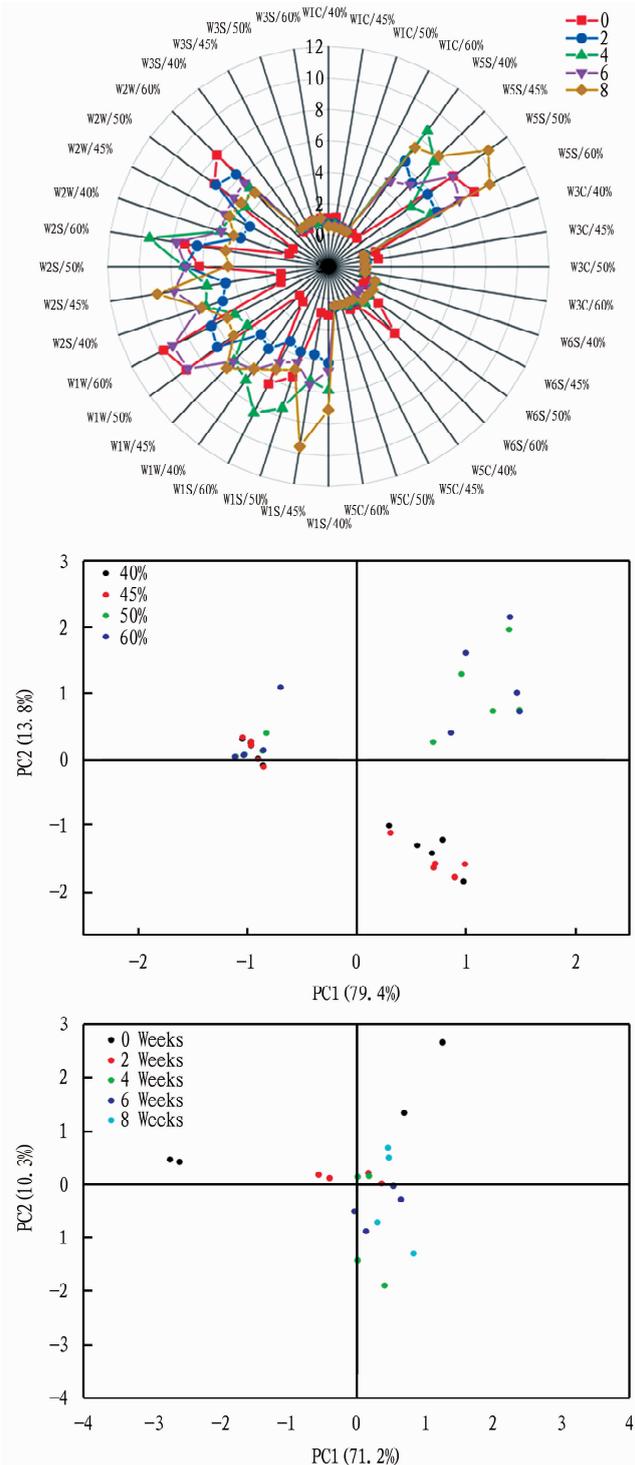


Fig. 8 Effect of different moisture content on the flavour of Sichuan sausage

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