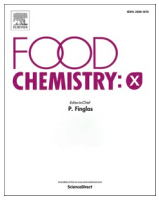
Food Chemistry: X 24 (2024) 101865

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Effect of 6-gingerol on oxidative stability and quality characteristics of mutton meatballs during refrigerated storage

Jiamei Li [a,b], Lijie Wang [a], Hongyan Mu [d], Geyi Ren [a,c], Mengyao Ge [a], Juan Dong [a,b,*], Qingling Wang [a,b], Jingtao Sun [a,c,*]

[a] School of Food Science, Shihezi University, Shihezi, Xinjiang 832003, China
[b] Key Laboratory of Agricultural Product Processing and Quality Control of Specialty (Co-construction by Ministry and Province), School of Food Science and Technology, Shihezi University, Shihezi, Xinjiang 832000, China
[c] Key Laboratory for Food Nutrition and Safety Control of Xinjiang Production and Construction Corps, School of Food Science and Technology, Shihezi University, Shihezi, Xinjiang 832000, China
[d] College of Food Science and Engineering, Qingdao Agricultural University, Changcheng Rd. 700#, Qingdao, Shandong Province 266109, China

ARTICLE INFO

Keywords:
6-gingerol
Cold storage
Physicochemical properties
Mutton meatballs
Oxidation

ABSTRACT

Meat products are high in nutrients but easily spoiled; adding antioxidants is the most straightforward and efficient approach in the food industry. In this study, different concentrations of 6-gingerol (6-GG) were added to refrigerated (4°C) mutton meatballs to evaluate the effect of 6-GG on their oxidative stability and quality characteristics. The results demonstrated that 6-GG prevented the increase in protein carbonyl content and decreased the loss of protein sulfhydryl content as storage times increased. Moreover, the thiobarbituric acid reactive substances (0.4643 mg MDA/kg) in the 0.0440% 6-GG treatment group did not exceed the pertinent fresh meat standards on day 7. The texture, water-holding capacity, and microstructure of the meatballs were also improved, suggesting that the addition of 6-GG is a feasible strategy to improve the quality of meatballs and meat products.

1. Introduction

Ready-made meatballs are becoming more and more popular among consumers because of their excellent, juicy flavor and nutritious value (Aviles et al., 2020). However, the proteins in meatballs can deteriorate during processing and storage due to exposure to reactive oxygen species, which leads to a reduction in quality (Domínguez et al., 2019; Saldaña et al., 2021). This discourages customers from purchasing these products. Antioxidant addition is a key method for reducing lipid and protein oxidation (Gutiérrez-del-Río et al., 2021). Antioxidants can be synthesized or found naturally. However certain artificial antioxidants are hazardous (Cenci-Goga et al., 2020). Therefore, current research has focused on the use of natural antioxidants as a substitute for synthetic antioxidants (Šimat et al., 2023).

Ginger is widely used in meat products, and 6-gingerol (6-GG) is its main bioactive component. The bioactivities of 6-GG include antioxidant (Si et al., 2018), anti-inflammatory (Alsahli et al., 2021), and anti-obesity (Jiao et al., 2022) effects both *in vitro and in vivo*. These properties give 6-GG a wide range of biological uses. Si et al. (2018) found that 6-GG trapped free radicals to prevent lipid peroxidation. The hydroxyl groups in 6-GG have redox characteristics and have lengthy hydrocarbon side chains, which makes them hydrophobic antioxidants. Mi et al. (2017) combined perilla oil and 6-GG in the surimi of grass carp. They reported that 6-GG not only prevented lipid and protein oxidation but also inhibited microbial growth during cold storage. Mi, Guo and Li (2016) evaluated the feasibility of using 6-GG to maintain the edible quality of refrigerated red drum fillets. The important point of these reports was that the 6-GG could prevent the fish from oxidizing too soon. The effect of 6-GG on the oxidative stability of poultry meat products has never been investigated. Therefore, it is important to valuate 6-GG as a new ingredient to improve the quality of poultry meat products. Significantly, 70%-75% of the total muscle mass is composed of water (Mi et al., 2017) and the strong correlation between meat quality and water content emphasizes the significance of evaluating changes in the water content of food items as a measure of their quality and physicochemical properties during storage (Wang et al., 2024; Xu et al., 2017).

The current study explored the effects of different concentrations of 6-GG (0.0088%, 0.0176%, and 0.0440%) on the oxidation of proteins

* Corresponding authors at: School of Food Science, Shihezi University, No. 211, North 4th Road, Xinjiang Autonomous Region, Shihezi, China.
E-mail addresses: dongjuanvv@126.com (J. Dong), 108826217@qq.com (J. Sun).

https://doi.org/10.1016/j.fochx.2024.101865
Received 8 August 2024; Received in revised form 17 September 2024; Accepted 27 September 2024
Available online 2 October 2024

and lipids in mutton meatballs during refrigeration. Moreover, the moisture distribution and content, color, pH, and gel properties of the mutton meatballs were investigated, and their microstructure was analyzed. The correlation between low-frequency nuclear magnetic resonance (LF-NMR) parameters and meatball quality parameters was studied using Pearson correlation. The current approach sought to establish a theoretical foundation for the storage and preservation of pre-made mutton meatballs as well as to ascertain the optimal amount of 6-gingerol to be applied to meat products.

2. Materials and methods

2.1. Materials and chemicals

Fresh leg of mutton and mutton tail fat were purchased from the Xinjiang Western Pastoral Co. in Shihezi, Xinjiang, China. Salt and potato starch were purchased from the Friendship Supermarket (Shihezi, China). 6-gingerol (6-GG, purity ≥98%, CAS number:23513-14-6) was sourced from Chengdu Preflex Technology Development Co., Ltd. (Chengdu, China). Compound phosphates used were of food grade, Anhydrous ethanol was acquired from Tianjin Fuyu Fine Chemical Co., Ltd., China, other chemicals used were of analytical grade.

2.2. Preparation of pre-made mutton meatballs

Mutton meatballs were prepared with slight modification of the method of Zwolan et al. (2020). The base mince was prepared by grating fresh lean mutton hind leg and sheep tail fat using a simple recipe as follows: 85% lean mutton hind leg, 15% sheep tail fat. The amounts of salt (1.6%), potato starch (5%), composite phosphate (0.3%) and ice water (16%) added were calculated with reference to the weight of the base mince. Three independent batches of mutton meatballs were made using this base mince, each batch was divided into four different treatment groups (Each group containing 40 mutton meatballs): Mb0 (no 6-gingerol added), Mb1 (0.0088% of 6-gingerol added), Mb2 (0.0176% of 6-gingerol added) or Mb3 (0.0440% of 6-gingerol added). Ice water was mixed with minced mutton meat added with other excipients in three times, and made into mutton meatballs. Each meatball was approximately 30 mm in diameter and weighed around 20 g. The meatballs were heated in a water bath at 80°C for 20 min, cooled to room temperature for 30 min, dried slightly on the surface, wrapped in plastic wrap, and stored at 4°C for 0, 1, 2, 3, 4, 5, 6, and 7 d, respectively (the meatballs of the 0th d was was taken out after 4 h of storage at 4°C for the determination of the relevant indexes). The amount of 6-gingerol added was set according to the amount of antioxidants used in meat products as specified in GB 2760-2014.

2.3. Determination of carbonyl content

According to the method described by Ren et al. (2022), myofibrillar protein (MP) was extracted. The carbonyl content were measured according to Wang et al. (2023). 2 mL of protein sample solution (2 mg/mL) was combined with 2 mL of 10 mol/L 2,4-dinitrophenylhydrazine (DNPH, dissolved in 2 mol/L HCl). The mixture was then allowed to react for 1 h at room temperature, being vortexed once every 15 min, and was protected from light; at the end of the reaction, 5 mL 20% trichloroacetic acid (TCA) was added to the reaction, and then centrifuged at 11000 *g* for 10 min after mixing and the supernatant was discarded. After the reaction, the precipitate was washed twice with 1 mL of anhydrous ethanol-ethyl acetate (1:1 = v:v) mixture to eliminate unreacted reagents. Subsequently, 3 mL of 6 mol/L guanidine hydrochloride was added to the precipitate, which was then dissolved in a water bath at 37°C for 15 min. The resulting mixture was centrifuged at 11000 *g* for 5 min to discard insoluble material. The supernatant was collected and its absorbance was measured at 370 nm. The carbonyl content was calculated using a molar extinction coefficient of 22,000 mol/L^{-1} cm^{-1}.

2.4. Determination of total sulfhydryl group contents

According to the method described by Ren et al. (2022), MP was extracted. The total sulfhydryl group contents were measured according to Yang, Liu, et al. (2023). Took 1 mL of protein sample solution (2 mg/mL), added 8 mL of Tris-glycine urea solution (pH = 8, 10.4 g Tris, 6.9 g glycine, 1.2 g EDTA, 8 mol/L urea per liter of this solution)), vortexed, shook well, and centrifuged at 11000 *g* for 20 min to remove insoluble proteins. Then, 0.5 mL of 10 mmol/L Ellman's reagent (DTNB, 2-nitrobenzoic acid, pH 8.0) was added. The reaction was conducted at room temperature for 30 min, and the absorbance value was measured at 412 nm. The molar extinction coefficient of 13,600 mol/L^{-1} cm^{-1} was applied to calculate the sulfhydryl group content.

2.5. Thiobarbituric acid reactive substances (TBARs)

Referring to the method of Zhao et al. (2022)with slight modification. The samples (5 g) were added into 30 mL 7.5% TCA (containing 0.1% EDTA) and homogenized for 30 s using a homogenizer (HU Analyzer-HR-500, China) at 17600 g. The mixture was then filtered through qualitative filter paper. Subsequently, 2 mL of the filtrate was combined with 2 mL of 0.02 mol/L thiobarbituric acid (TBA) (ready-to-use) and heated at 90°C for 30 min. After rinsing with cold water to room temperature, the absorbance was measured at 532 nm and 600 nm. The results were expressed as the mass of malondialdehyde per kilogram of meat sample, and the TBARs values were calculated using the following formula:

$$\text{TBARS (mg/kg MDA)} = \frac{(A_{532} - A_{600}) \times 72.6 \times 100}{155 \times m} \quad (1)$$

where A_{532} is the absorbance at 532 nm; A_{600} is the absorbance at 600 nm; m is the weight of mutton meatballs sample, g; 72.6 is the molar mass of malondialdehyde (MDA), g/mol; 155 is the molar extinction coefficient.

2.6. Gel strength

The gel strength of the meatballs was determined using a mass spectrometer. After the meatballs were removed from the refrigerator and left at room temperature for 30 min, they were cut into cylinders with a diameter of 6 mm and a height of 5 mm for the determination of the gel strength, and the probe was selected as P/0.5, with the following conditions: pre-test speed of 2 mm/s; test speed of 0.5 mm/s; post-test speed of 2 mm/s; downward displacement of 5 mm; and trigger force of 15 g. The gel strength was measured by the plasmapheresis (Ren et al., 2023).

2.7. Water holding capacity (WHC)

The weighed meatballs (W_1) were wrapped in filter paper and placed in a centrifuge tube and centrifuged at 8800 *g* for 10 min (4°C) at the end of the centrifugation, they were removed and weighed (W_2) (Barbut, 2024). The WHC calculation formula is as follows:

$$\text{WHC (\%)} = \frac{W_2}{W_1} \times 100 \quad (2)$$

2.8. pH

5.0 g of sample was weighed and chopped, 50 mL of distilled water was added, and the sample was homogenized by a homogenizer at a high speed (22,000 g) for 30 s and then leave to stand for 120 s. The pH meter was adjusted to zero and then placed in the sample, and the pH value was recorded after the value stabilized (Lian et al., 2023).

2.9. Color

Took five random spots on the surface of the meatball. L*, a* and b* of the gel were determined using a NR60CP High-Quality Colorimeter (Measuring aperture: 8 mm; Color space: CIE LAB).

2.10. Cooking loss

Weigh the mass of the meatballs before cooking and write it as m_1; took the frozen meatballs out of the refrigerator at 4°C, standed them at room temperature for 30 min, dried the water stains on the surface of the meatballs with absorbent paper and weighed them as m_2 (Zhong et al., 2022), and then calculated the rate of cooking loss according to the following formula:

$$\text{Cooking loss}\ (\%) = \frac{m_1 - m_2}{m_1} \times 100 \quad (3)$$

2.11. Texture profile analysis

The meatballs were removed from the refrigerator in advance and placed at room temperature to rewarm for 30 min before being subjected to texture testing using a Texture Analyzer (TA-XT plus, Shanghai Precision Instruments Co., Ltd.) with the following parameters: probe P/50, pre-test speed of 5.00 mm/s, mid-test speed of 2.00 mm/s, post-test speed of 5.00 mm/s, compression degree of 50%, trigger force of 5.0 g, and 5 s interval (Hu et al., 2017).

2.12. Determination of moisture distribution and content

Water distribution in meatballs was determined using spin relaxation time (T_2) with a MesNMI20clear magnetic resonance analyzer (Suzhou Niumag Analytical Instrument Co., Ltd.). ples were measured after the meatballs were allowed to stand at room temperature (25°C) for 30 min according to Lian et al. (2023) with the following parameters: measurement temperature of 32°C, repetition time of 11 ms; echo time (time between 90° and 180° pulses) of 200 μs; number of echoes of 5000 and number of scans = 4. Magnetic Resonance Imaging (MRI) was acquired using an inversion recovery (IR) imaging sequence.TR = 3500 ms, TE = 50 ms. Acquired grayscale images were pseudo-colored by the MRI processing software (Niumag Electric Co., Shanghai, China) to obtain the proton density map. Visualize the moisture content.

2.13. Microstructure observation

The meatball samples were cut into uniformly sized blocks (3 × 3 × 3 mm^3), fixed by immersion in 2.5% glutaraldehyde, placed in a refrigerator at 4°C for 48 h protected from light, and then rinsed three times with 0.01 mol/L PBS (pH 7.4) at room temperature to remove the glutaraldehyde solution. Then the samples were dehydrated and freeze-dried with 30%, 50%, 70%, and 100% ethanol solutions, respectively, in volume fraction. Platinum was sprayed on the samples, and then the microstructure was observed using a scanning electron microscope at (SU800, Tokyo, Japan) 400 × (Zhao et al., 2024).

2.14. Statistical analysis

At day 0, three independent batches of mutton meatballs were prepared, with a total of four treatments per batch. Each batch of mutton meatballs was measured and analyzed for relevant traits at least three times, data were analyzed by one-way analysis of variance (ANOVA) using SPSS (version 26.0, SPSS Statistics, IBM, New York, NY, USA) with results expressed as mean ± standard deviation (X ± SD), and all data were analyzed for significance of difference ($p < 0.05$) using Duncan's new complex polarity method. Graphs were generated using Origin (version 2021, Origin Lab, Hampton, Massachusetts, USA) and Omicstudio (https://www.omicstudio.cn/Home).

3. Results and discussion

3.1. Carbonyl content

Carbonyl groups and their derivatives are formed when side chains of amino acids undergo oxidation due to peptide bond breaking in proteins. As a result, the content of carbonyl can reveal the extent to which the protein has been oxidized (Ren et al., 2022). Fig. 1A depicts the changes in the protein carbonyl content of mutton meatballs during cold storage following treatment with various 6-GG concentrations. When compared to day 0, the carbonyl content in the various treatment groups increased significantly ($p < 0.05$) up to day 7. This indicated that the proteins were significantly oxidized during cold storage. However, the carbonyl content of the 6-GG treatment groups (Mb1, Mb2, and Mb3) was lower than that of the control group (Mb0) at the same storage period time. This result demonstrated that 6-gingerol effectively inhibited the formation of carbonyls in meat. Polyphenols prevent protein oxidation by scavenging free radicals and chelating metal ions, shielding proteins from hydroxyl radical damage. Huang et al. (2022) demonstrated that the addition of 0.5% mulberry polyphenols could significantly protect proteins from oxidative attack, thereby inhibiting the formation of protein carbonyls.

3.2. Total sulfhydryl group contents

Sulfhydryl groups in proteins are converted to disulfide groups upon oxidation. Therefore, the change in total sulfhydryl group content can be used to determine the extent of protein oxidation during cold storage (Zhong et al., 2022). It is evident from Fig. 1B that with the increase in cold storage time, the total sulfhydryl group content of each treatment group decreased ($p < 0.05$). The total sulfhydryl content of the protein was lost as a result of these changes exposing the -SH groups therein and oxidizing them to S-S (Yang, Yan, & Xie, 2023). Particularly, the total sulfhydryl content of the 6-GG treatment groups (Mb1, Mb2, and Mb3) was higher than the control group at the same storage time, indicating that 6-GG was able to reduce the loss of the total sulfhydryl content in the protein during cold storage.

3.3. Thiobarbituric acid reactive substances (TBARs)

The oxidation of fatty acid produces aldehydes, which produce colored compounds when combined with thiobarbituric acid (TBA). Therefore, variations in TBAR values can be used to determine the extent of fat oxidation during cold storage (Nuerjiang et al., 2023). Moreover, the TBAR value can also indicate the freshness of meatballs (0.202-0.664 mg MDA/kg for fresh meat; 0.664-1.000 mg MDA/kg for second-fresh meat; and > 1.000 mg MDA/kg for spoiled meat, where MDA represents malondialdehyde) (Sharma et al., 2017; Yang et al., 2020). The TBAR values of each treatment group demonstrated a significant ($p < 0.05$) increasing trend with an increase in cold storage time (Fig. 1C). This observation might be explained by the oxidation of fatty acids that occurred during cold storage, which raised the amount of MDA. As a result of the reaction with TBA, more red compounds were formed, which enhanced the TBAR values (Šimat et al., 2023). During the same storage period, the TBAR values of the 6-GG treatment groups (Mb1, Mb2, and Mb3) were significantly ($p < 0.05$) lower than that of the control group, suggesting that 6-GG effectively reduced lipid oxidation. Furthermore, the TBAR value of Mb0 was found to be 1.0605 mg MDA/kg on day 3 of cold storage, 1.1596 mg MDA/kg on day 5 of refrigeration for Mb1, and 1.1639 mg MDA/kg on day 6 of cold storage for Mb2, indicating that the addition of 6-GG prolonged the shelf-life of the pre-made mutton meatballs. However, the Mb3 meatballs remained fresh after 7 days of refrigeration, most likely because of the antioxidant characteristics of 6-GG and the efficient suppression of lipid oxidation

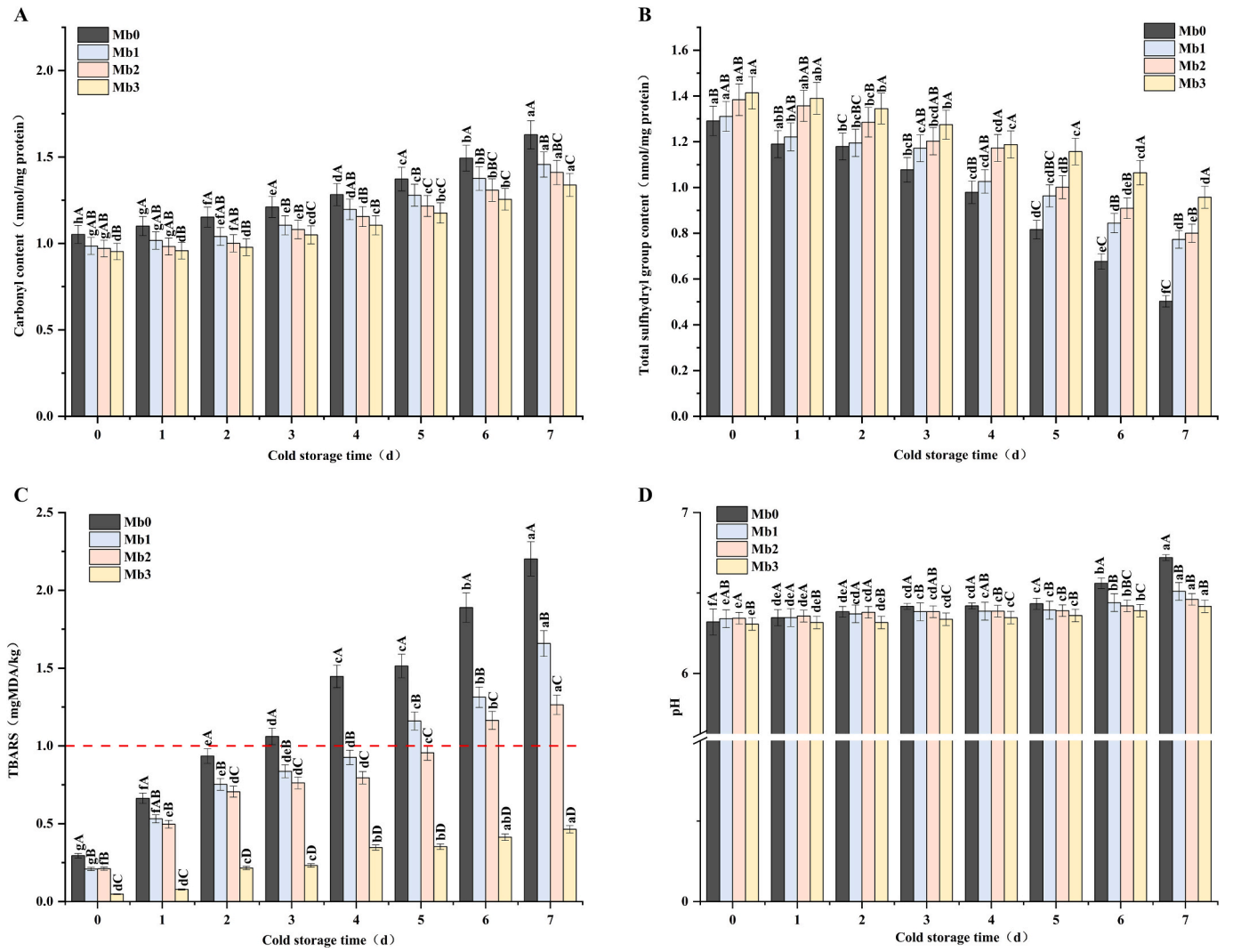


Fig. 1. Changes in the carbonyl contents (A), total sulfhydryl content (B), TBARs values (C) and pH (D) of mutton MP during cold storage.
Note: The Mb0, Mb1, Mb2, and Mb3 treatment groups represent the addition of 0, 0.0088, 0.0176, and 0.0440% of 6-gingerol, respectively; letters (a-h) represent significant ($p<0.05$) differences between different cold storage times in the same treatment group; letters (A-C) represent significant ($p<0.05$) differences between different treatment groups for the same cold storage time.

Table 1
Changes in the gel properties of mutton meatballs during cold storage.

	Sample	Cold storage time(d)							
		0	1	2	3	4	5	6	7
Gel strength (g)	Mb0	1149.36 ± 92.18^{aD}	1137.28 ± 26.19^{aD}	1048.14 ± 46.62^{bD}	972.96 ± 19.23^{bcD}	906.64 ± 50.81^{cD}	820.58 ± 14.50^{dD}	788.37 ± 18.42^{eD}	724.17 ± 11.06^{fD}
	Mb1	1272.04 ± 4.46^{aC}	1262.48 ± 60.15^{aC}	1224.45 ± 20.53^{abC}	1154.83 ± 38.80^{bcC}	1078.87 ± 53.23^{cC}	1079.64 ± 61.01^{cC}	1005.46 ± 28.51^{dC}	935.08 ± 21.29^{eC}
	Mb2	1470.32 ± 10.62^{aB}	1405.72 ± 69.52^{abB}	1395.29 ± 36.41^{abB}	1357.72 ± 36.22^{bcB}	1324.36 ± 83.57^{bcB}	1300.94 ± 18.06^{cB}	1288.31 ± 57.20^{dB}	1246.27 ± 14.78^{dB}
	Mb3	1663.42 ± 38.87^{aA}	1651.07 ± 40.34^{aA}	1619.79 ± 29.61^{abA}	1610.30 ± 45.50^{abA}	1549.18 ± 66.44^{bA}	1391.26 ± 55.48^{cA}	1306.88 ± 38.41^{dA}	1273.12 ± 11.41^{dA}
WHC (%)	Mb0	94.73 ± 0.27^{aC}	94.47 ± 0.33^{aC}	93.80 ± 0.20^{bC}	93.67 ± 0.13^{bcC}	93.23 ± 0.43^{cC}	92.27 ± 0.13^{dC}	91.22 ± 0.11^{eC}	89.17 ± 0.23^{fC}
	Mb1	95.53 ± 0.27^{aB}	95.13 ± 0.13^{bB}	94.80 ± 0.20^{cB}	94.61 ± 0.03^{cB}	94.20 ± 0.20^{dB}	93.60 ± 0.40^{eB}	93.07 ± 0.18^{efB}	92.56 ± 0.22^{fB}
	Mb2	95.60 ± 0.40^{aAB}	95.40 ± 0.20^{aAB}	95.33 ± 0.33^{abA}	94.73 ± 0.33^{bcB}	94.33 ± 0.33^{cB}	93.67 ± 0.33^{dB}	93.13 ± 0.20^{dB}	92.83 ± 0.27^{fB}
	Mb3	96.33 ± 0.67^{aA}	95.93 ± 0.13^{abA}	95.60 ± 0.12^{bcA}	95.60 ± 0.20^{bcA}	95.40 ± 0.20^{bcA}	95.07 ± 0.13^{cA}	94.88 ± 0.39^{cdA}	94.57 ± 0.21^{dA}

Note: The Mb0, Mb1, Mb2, and Mb3 treatment groups represent the addition of 0, 0.0088, 0.0176, and 0.0440% of 6-gingerol, respectively; WHC represent water holding capacity. Same as below.

reactions in the meatballs at higher concentrations (Yang, Yan, & Xie, 2023), indicating that the optimal concentration of 6-GG to add was 0.0440%.

3.4. Gel strength and WHC

Table 1 shows the changes in the gel strength and WHC of meatballs during cold storage in different 6-GG treatment groups (Mb1, Mb2, and Mb3). Gel strength is one of the important indicators of meatball quality and is related to the structural integrity of the meatballs (Feng et al., 2023). As the duration of cold storage increased, lipid and protein oxidation caused a significant ($p < 0.05$) decrease in the gel strength for every treatment group. However, the gel strength of Mb0 was significantly lower than that of the 6-GG treatment groups (Mb1, Mb2, and Mb3) on the 7th day of cold storage. This showed that 6-GG prevented the gel strength of the mutton meatballs from decreasing, preserving their structural integrity and significantly enhancing the caliber of mutton meatballs kept in cold storage. These results corresponded with the results obtained by Xu et al. (2021). They showed that incorporating tannins, gallic acid, (−)-epigallocatechin gallate, and epigallocatechin into pork meatballs increased the gel strength of the meatballs and improved their overall quality.

The water content in the muscle directly affects the tenderness, juiciness, and texture of meatballs (Yao et al., 2023). Table 1 indicates that as the meatballs were stored for longer periods, reduced elasticity of meatballs when cut, their WHC was considerably reduced ($p < 0.05$). This could be because the structure of the meatballs suffered some oxidative damage as storage duration increased, which resulted in moisture loss and was consistent with the gel strength findings. However, the loss of water was effectively suppressed with an increase in the content of 6-GG. This may be due to the interaction between 6-gingerol and proteins as well as the tightness of the gel network of the protein complexes, which increased the number of water molecules bound to the proteins. Shen et al. (2024) found that the gel WHC was significantly increased when chicken MP was mixed with increasing concentrations (0.1, 0.3, 0.5, 0.7, and 1.0 wt%) of fibrous microfibrillated cellulose, rod microcrystalline cellulose, and needle nanocrystalline cellulose. Furthermore, the gel WHC increased as the amount of the four celluloses increased. This was attributed to the spatial site resistance of the cellulose and its interaction with the MP, which affected the relative defolding and MP aggregation rate, which led to a lower rate of protein aggregation than the denaturation rate. As a result, a denser and more homogeneous microstructure was formed, significantly enhancing the gel WHC ($p < 0.05$).

3.5. pH

The alterations in the pH of mutton meatballs with different 6-GG treatments during cold storage, are shown in Fig. 1D. Due to the formation of alkaline byproducts on oxidation of meatballs, the pH of each treatment group increased with an increase in storage time (Echegaray et al., 2018). Moreover, certain psychrophilic microorganisms grow and metabolize during the cold storage period, which produces alkaline byproducts, such as ammonia and trimethylamine, leading to high pH levels (Jogdand et al., 2023). Following 7 days of cold storage, the pH of the Mb0, Mb1, Mb2, and Mb3 groups were 6.72, 6.51, 6.46, and 6.42, respectively. Thus, the pH of the Mb0 group was significantly ($p < 0.05$) higher than the 6-GG treatment groups (Mb1, Mb2, and Mb3). This demonstrated that 6-GG preserved the meatball quality of the meatballs by inhibiting protein degradation.

3.6. Color

Color is a crucial visual feature in meat and meat products, which affects the purchase choices of consumers (Neethling et al., 2016). Table 2 shows that the L* values of all treatment groups, except the Mb3 group, increased significantly ($p < 0.05$) with an increase in storage time. The moisture oozing from the surface of the meatballs increased the reflection of light, which enhanced the brightness. The L* value of the 6-GG-treated groups was significantly ($p < 0.05$) lower than that of

Table 2
Color changes and cooking loss in mutton meatballs during cold storage.

Metric	Sample	Cold storage time(d)							
		0	1	2	3	4	5	6	7
L*	Mb0	63.71 ± 0.82^{cA}	64.06 ± 2.42^{bcA}	64.16 ± 1.76^{bcA}	64.36 ± 1.06^{bcA}	65.29 ± 1.13^{bA}	66.55 ± 1.63^{bA}	67.15 ± 0.71^{abA}	68.06 ± 1.58^{aA}
	Mb1	60.72 ± 2.04^{bB}	61.13 ± 1.27^{bB}	61.20 ± 1.55^{bB}	61.29 ± 1.54^{bB}	61.93 ± 1.29^{abB}	62.89 ± 0.99^{aB}	63.48 ± 1.62^{aB}	63.88 ± 0.88^{aB}
	Mb2	59.41 ± 0.46^{bC}	60.39 ± 1.76^{bB}	60.39 ± 1.98^{abB}	60.39 ± 1.05^{abB}	60.86 ± 1.04^{aB}	61.34 ± 0.83^{aC}	61.74 ± 1.02^{aC}	62.03 ± 1.38^{aC}
	Mb3	59.86 ± 1.23^{aBC}	60.11 ± 2.61^{aB}	60.16 ± 1.21^{aB}	60.27 ± 1.53^{aB}	60.46 ± 2.89^{aB}	61.49 ± 1.90^{aC}	61.57 ± 1.75^{aC}	61.74 ± 2.11^{aC}
a*	Mb0	8.38 ± 0.35^{aB}	8.23 ± 0.17^{abB}	8.26 ± 0.62^{abB}	8.14 ± 0.17^{abB}	7.67 ± 0.45^{bB}	7.64 ± 1.33^{bB}	7.57 ± 0.62^{bB}	6.98 ± 0.49^{bB}
	Mb1	8.95 ± 0.92^{aA}	8.70 ± 0.62^{aA}	8.59 ± 0.39^{aAB}	8.44 ± 0.48^{aA}	8.31 ± 0.85^{aAB}	7.82 ± 0.53^{bB}	7.79 ± 0.25^{bB}	7.48 ± 0.71^{bB}
	Mb2	9.02 ± 0.68^{aA}	9.09 ± 0.53^{aA}	8.71 ± 0.57^{abA}	8.62 ± 0.76^{abA}	8.57 ± 0.53^{abA}	8.00 ± 1.26^{bAB}	7.96 ± 0.36^{bB}	7.51 ± 1.02^{bB}
	Mb3	9.18 ± 0.21^{aA}	9.07 ± 0.84^{aA}	8.84 ± 0.87^{aA}	8.71 ± 0.93^{aA}	8.66 ± 0.68^{aA}	8.63 ± 0.39^{aA}	8.31 ± 0.47^{aA}	8.30 ± 0.34^{aA}
b*	Mb0	9.10 ± 0.26^{bB}	9.29 ± 0.24^{bB}	9.30 ± 0.36^{bA}	9.68 ± 0.19^{aBA}	9.80 ± 0.28^{aB}	9.83 ± 0.44^{aB}	9.87 ± 0.17^{aB}	10.05 ± 0.25^{aB}
	Mb1	9.30 ± 0.62^{bAB}	9.46 ± 0.38^{abAB}	9.43 ± 0.78^{abA}	9.89 ± 0.57^{abA}	9.97 ± 0.78^{aAB}	10.04 ± 0.55^{aB}	10.15 ± 0.69^{aB}	10.33 ± 0.50^{aB}
	Mb2	9.45 ± 0.39^{aAB}	9.50 ± 0.30^{aAB}	9.64 ± 0.67^{abA}	10.07 ± 0.84^{abA}	10.10 ± 0.61^{abAB}	10.15 ± 0.68^{abB}	10.37 ± 0.37^{aB}	10.67 ± 0.20^{aB}
	Mb3	9.61 ± 0.66^{dA}	9.66 ± 0.35^{cdA}	9.89 ± 0.82^{cdA}	10.11 ± 0.39^{cA}	10.37 ± 0.39^{bcA}	10.72 ± 0.37^{bA}	11.09 ± 0.23^{bA}	11.59 ± 0.59^{aA}
Cooking loss (%)	Mb0	8.47 ± 0.11^{dA}	8.66 ± 0.31^{acdA}	8.77 ± 0.02^{cdA}	8.90 ± 0.21^{cdA}	9.03 ± 0.15^{cdA}	9.14 ± 0.11^{cA}	9.34 ± 0.14^{bA}	9.81 ± 0.21^{aA}
	Mb1	7.25 ± 0.13^{cAB}	7.27 ± 0.17^{cB}	7.33 ± 0.06^{cB}	7.38 ± 0.17^{cB}	7.44 ± 0.16^{cAB}	7.49 ± 0.23^{bB}	7.88 ± 0.18^{abB}	8.02 ± 0.04^{aB}
	Mb2	7.11 ± 0.19^{abB}	7.16 ± 0.05^{abB}	7.22 ± 0.22^{abB}	7.28 ± 0.19^{abAB}	7.33 ± 0.11^{abB}	7.39 ± 0.16^{abB}	7.43 ± 0.38^{abC}	7.62 ± 0.13^{aC}
	Mb3	7.10 ± 0.10^{abB}	7.15 ± 0.21^{abB}	7.21 ± 0.09^{abB}	7.26 ± 0.16^{abB}	7.32 ± 0.12^{abB}	7.38 ± 0.13^{abB}	7.41 ± 0.22^{abC}	7.50 ± 0.12^{aC}

the Mb0 group due to the reduction in light reflection following the introduction of 6-GG to the meatballs. 6-GG also interacts with proteins to form a denser structure (Xu et al., 2018), which is with the previously obtained WHC results.

The a* values of every treatment group reduced as storage time increased, whereas b* values increased. These changes could be caused by the oxidation of myoglobin, which produced lighter-colored high-iron myoglobin after the meat exhibited more yellow tones (Neethling et al., 2016). On day 7, the a* value of the 6-GG treatment groups (Mb1, Mb2, and Mb3) was higher than that of Mb0 and close to that of Mb0 on day 0. This could be because the hydroxyl structure of the 6-GG rapidly combines with This may be due to 6-GG through its hydroxyl structure quickly and myoglobin radicals to form more stable substances, slowing down or stopping the chain reaction of protein oxidation radicals. Alternatively, the ability of the 6-GG to chelate metal ions and decompose peroxides prevented metal ions from catalyzing oxidation reactions (Jiang & Xiong, 2016; Li et al., 2020; Zhu et al., 2024), terminating the oxidation reaction, and preventing the color change caused by oxidation during storage, thereby contributing to color protection. Moreover, on day 0, the a* and b* values of the 6-GG treatment groups (Mb1, Mb2, and Mb3) were higher than the Mb0. These color variations were influenced by the color of the 6-GG itself. Similar results were reported by Fernández-López et al. (2023). They showed that the a* and b* values of the samples changed to varied degrees when different kinds of beet juice were added. Mixed minced pork and beef with boiled beet juice had greater a* and b* values, but mixed minced pork and beef with hydrated beet juice had higher a* and lower b* values.

3.7. Cooking loss

Cooking loss is a crucial quality factor that directly impacts the yield and quality of processed meat products. This loss can be a reflection of both the type and functional properties of the muscle proteins (Pang et al., 2020). Table 2 lists the changes in the cooking loss of mutton meatballs during cold storage upon adding different amounts of 6-GG. An increase in cooking loss from 8.47% to 9.14% in the Mb0 group was statistically significant ($p < 0.05$). The enhanced in water loss augmented the light reflection and increased the L* value, which was consistent with the above results. However, there was only a minor difference in the cooking loss in the polyphenol treatment groups (Mb1, Mb2, and Mb3), likely because the interaction of polyphenols with proteins formed a good three-dimensional gel network structure, which enhanced water retention properties (Xu et al., 2021). This was consistent with the results of the WHC for mutton meatballs during cold storage.

3.8. Texture

One of the main characteristics of meat products impacting consumer choice is texture (Nishinari et al., 2024). Table 3 shows that the hardness, springiness, cohesiveness, chewiness, and resilience of meat were significantly ($p < 0.05$) affected by the duration of cold storage. The hardness of the treatment groups considerably reduced ($p < 0.05$) as the length of cold storage increased, owing to the oxidative disruption of proteins and connective tissues as well as the autolytic and microbiological degradation of muscles (Coombs et al., 2018). Initially, the hardness of Mb0 found to be 1488.422 g. Following 7 days of cold storage, the hardness of each treatment group (Mb0、Mb1、Mb2、Mb3) found to be 869.025, 1887.870, 1998.123 and 2621.045 g, respectively. This suggested that 6-GG not only significantly ($p < 0.05$) prevented the decrease in hardness in the meatballs but also significantly ($p < 0.05$) improved their hardness. Similar results were reported by Ramírez-Suárez and Xiong (2003). They demonstrated that the interaction between meat products and soy proteins during heating promoted the formation of a gel matrix, which hardened the soy meat

Table 3
Texture changes in mutton meatballs during cold storage.

	Cold storage time/d	Mb0	Mb1	Mb2	Mb3
Hardness	0	1488.422 ± 64.781^{aC}	2458.132 ± 238.088^{aB}	2612.830 ± 119.966^{aB}	3412.370 ± 147.713^{abA}
	1	1559.853 ± 43.001^{aC}	2648.067 ± 51.059^{aB}	2628.069 ± 124.570^{aB}	3673.803 ± 276.473^{aA}
	2	1291.038 ± 78.560^{bC}	2618.800 ± 165.316^{aB}	2615.700 ± 68.278^{aB}	3683.134 ± 50.353^{aA}
	3	1280.486 ± 33.333^{bC}	2498.035 ± 72.195^{aB}	2518.648 ± 118.048^{aB}	3351.106 ± 192.951^{bcA}
	4	1243.611 ± 198.050^{bC}	1976.967 ± 160.181^{bB}	2214.270 ± 98.772^{bB}	3069.970 ± 78.232^{cA}
	5	982.327 ± 66.876^{cD}	1939.261 ± 44.667^{bC}	2153.748 ± 147.339^{bB}	2704.090 ± 141.974^{dA}
	6	893.141 ± 31.770^{dD}	1907.040 ± 75.384^{bC}	2097.040 ± 97.083^{cB}	2639.657 ± 121.045^{dA}
	7	869.025 ± 154.144^{dD}	1887.870 ± 65.112^{bC}	1998.123 ± 74.124^{cdB}	2621.045 ± ±138.914^{dA}
Springiness	0	0.842 ± 0.047^{abC}	0.869 ± 0.017^{aB}	0.879 ± 0.033^{aB}	0.931 ± 0.026^{abA}
	1	0.850 ± 0.045^{aC}	0.863 ± 0.003^{abB}	0.876 ± 0.007^{abB}	0.944 ± 0.054^{aA}
	2	0.840 ± 0.017^{abC}	0.858 ± 0.023^{abB}	0.873 ± 0.038^{abB}	0.909 ± 0.023^{abA}
	3	0.840 ± 0.023^{abC}	0.851 ± 0.025^{bB}	0.866 ± 0.030^{bB}	0.899 ± 0.022^{bA}
	4	0.822 ± 0.013^{bC}	0.840 ± 0.023^{cBC}	0.857 ± 0.039^{bcB}	0.900 ± 0.072^{bA}
	5	0.809 ± 0.009^{bC}	0.838 ± 0.056^{cBC}	0.850 ± 0.035^{cB}	0.903 ± 0.043^{bA}
	6	0.794 ± 0.015^{bC}	0.832 ± 0.016^{cB}	0.847 ± 0.014^{cB}	0.894 ± 0.011^{bA}
	7	0.776 ± 0.008^{cD}	0.812 ± 0.024^{dC}	0.840 ± 0.023^{cB}	0.881 ± 0.031^{bA}
Cohesiveness	0	0.553 ± 0.084^{aB}	0.611 ± 0.081^{aAB}	0.605 ± 0.023^{aB}	0.726 ± 0.012^{aA}
	1	0.497 ± 0.036^{aC}	0.551 ± 0.031^{abB}	0.582 ± 0.007^{abB}	0.709 ± 0.011^{abA}
	2	0.487 ± 0.016^{abC}	0.576 ± 0.016^{abB}	0.575 ± 0.019^{abcB}	0.653 ± 0.045^{bcA}
	3	0.475 ± 0.047^{abB}	0.519 ± 0.041^{bB}	0.575 ± 0.045^{abcA}	0.652 ± 0.035^{cA}
	4	0.468 ± 0.038^{abC}	0.518 ± 0.038^{bBC}	0.550 ± 0.025^{bcB}	0.638 ± 0.053^{bcA}
	5	0.399 ± 0.066^{bC}	0.513 ± 0.021^{bB}	0.525 ± 0.027^{cB}	0.605 ± 0.035^{cA}
	6	0.378 ± 0.065^{bC}	0.508 ± 0.018^{bB}	0.517 ± 0.017^{cB}	0.596 ± 0.015^{cA}
	7	0.369 ± 0.036^{bC}	0.496 ± 0.013^{bB}	0.514 ± 0.020^{cB}	0.593 ± 0.014^{cA}
Chewiness	0	958.850 ± 86.047^{aD}	1543.167 ± 84.017^{aC}	1795.777 ± 90.033^{aB}	2302.610 ± 64.026^{aA}
	1	871.049 ± 81.045^{bC}	1514.443 ± 112.003^{aB}	1494.288 ± 95.007^{bB}	2199.404 ± 169.054^{bA}
	2	751.383 ± 92.017^{cD}	1285.023 ± 118.023^{bC}	1517.175 ± 73.038^{bB}	1991.043 ± 98.023^{bA}
	3	672.152 ± 53.023^{dC}	1234.760 ± 102.025^{bB}	1391.022 ± 76.030b^{cB}	1790.537 ± 79.022^{cA}

(continued on next page)

Table 3 (*continued*)

	Cold storage time/d	Mb0	Mb1	Mb2	Mb3
	4	681.892 ± 58.013^{dD}	1103.789 ± 90.023^{cC}	1295.124 ± 81.039^{dB}	1540.842 ± 72.072^{dA}
	5	608.355 ± 97.009^{eC}	977.940 ± 115.056^{dB}	1100.764 ± 77.035^{eAB}	1183.236 ± 110.043^{eA}
	6	596.613 ± 52.022^{eD}	978.474 ± 73.210^{dC}	1096.987 ± 83.277^{eAB}	1169.663 ± 121.011^{eA}
	7	593.561 ± 76.017^{eC}	938.397 ± 97.615^{dB}	1072.905 ± 77.940^{eB}	1152.139 ± 55.129^{eA}
Resilience	0	0.261 ± 0.002^{aB}	0.275 ± 0.023^{aAB}	0.275 ± 0.008^{aAB}	0.299 ± 0.019^{aA}
	1	0.244 ± 0.011^{aC}	0.262 ± 0.007^{aB}	0.267 ± 0.008^{abAB}	0.277 ± 0.007^{abA}
	2	0.196 ± 0.007^{bC}	0.256 ± 0.005^{aAB}	0.246 ± 0.004^{bcB}	0.272 ± 0.019^{abcA}
	3	0.164 ± 0.021^{cC}	0.217 ± 0.027^{bB}	0.238 ± 0.021^{cdAB}	0.274 ± 0.029^{abA}
	4	0.124 ± 0.022^{dC}	0.195 ± 0.023^{bcB}	0.218 ± 0.019^{dB}	0.259 ± 0.015^{bcA}
	5	0.117 ± 0.013^{dC}	0.180 ± 0.019^{cB}	0.215 ± 0.012^{dA}	0.238 ± 0.017^{cA}
	6	0.118 ± 0.003^{dC}	0.178 ± 0.011^{cB}	0.214 ± 0.004^{dA}	0.225 ± 0.007^{cA}
	7	0.103 ± 0.018^{dC}	0.171 ± 0.012^{cB}	0.209 ± 0.016^{dA}	0.221 ± 0.010^{cA}

products.

The springiness and cohesiveness of the meatballs decreased significantly ($p < 0.05$) with an increase in the duration of cold storage. This indicated that the meatballs deteriorated in quality during cold storage. However, the inclusion of 6-GG led to a considerable increase ($p < 0.05$) in both attributes. Meat chewiness ultimately determines cohesion, resilience, hardness, and springiness. Although these properties decreased significantly ($p < 0.05$) with an increase in cold storage time, they increased with the addition of 6-GG (Baugreet et al., 2018). Thus, chewiness and resilience have the same trend as hardness. These findings suggest that during cold storage, 6-GG inhibited the deterioration of textural properties and improved the quality of meatballs. Hence, the addition of 6-GG improved the textural properties of meatballs, which is consistent with the results of WHC and cooking loss.

3.9. Moisture distribution and content

There are three types of water in the meatballs bound water, fixed, and free water. A decreasing order of bond strength was reported between these three types of water and meatballs. The relaxation times of bound water in LF-NMR were found to be 0–1 ms for T_{2a} and 1-10 ms for T_{2b}. For fixed water, T_{21} (10-100 ms) was present in the complex outer lattice of the myogenic fibers and accounted for the majority of the total muscle water. On the other hand, the binding strength of free water, T_{22}, which was most susceptible to water loss, was found to be 100-1000 ms (Zou et al., 2022). Fig. 2A shows the changes in the water distribution of mutton meatballs during cold storage at different 6-GG treatments. The peak areas represent the contents of T_{2a}, T_{2b}, T_{21}, and T_{22}, respectively. The levels of bound water were nearly the same, while those of the fixed water were significantly lower ($p < 0.05$), and those of free water were significantly enhanced ($p < 0.05$) in all treatment groups during the cold storage. These results indicated that during cold storage, the fixed water in the meatballs gradually converted into free water. Moreover, the polyphenol treatment groups (Mb1, Mb2, and Mb3) possessed higher bound and fixed water levels and less free water levels in comparison to the Mb0 group throughout the storage period. Further, the declining trend of fixed water was less noticeable as the polyphenol content increased. This was explained by the fact that 6-GG interacted with the sarcomeric proteins to form a three-dimensional network and prevented oxidative damage to the protein structure (Luo et al., 2023).

MRI is a non-invasive imaging method that visualizes the interior structure, such as the distribution of water in mutton meatballs, non-destructively using T1-weighting. The red color in the figure represents high levels of hydrogen proton density, while the blue color indicates low levels of hydrogen proton density (Yang, Yan, & Xie, 2023). Fig. 2B shows the MRI of mutton meatballs during cold storage at various 6-GG treatments. A rapid decrease in the hydrogen proton density was observed in the Mb0 group during cold storage, while a slow decrease was observed in the polyphenol treatment groups. A gradual decline in concentration was observed as the concentration of 6-GG increased. The findings indicated that 6-GG successfully preserved the water-holding capacity (WHC) of the mutton meatballs and that drip loss was caused by the slow ejection of water molecules from the interior to the surface (Wang et al., 2020). Therefore, the results of LF-NMR and MRI for the distribution of water were consistent.

3.10. Microstructure

Fig. 2C shows the changes in the microstructure of mutton meatballs during cold storage at different 6-GG treatments. The Mb0 showed pores and fissures and a loose, rough structure on day 0. However, the mutton groups that received the polyphenol treatment (Mb1, Mb2, and Mb3) showed a more homogeneous and smooth structure with fewer holes, with Mb3 having the fewest pores and the densest structure. This demonstrated that adding the right dosage of 6-GG improved the microstructure of the mutton meatballs. The distinct structure of 6-GG, which promoted interaction with MP and resulted in structural alterations in the protein, was responsible for the effects of 6-GG on protein structure and gel properties under various environmental conditions. This, in turn, led to the formation of complex covalent cross-linking patterns between proteins and proteins and between proteins and polyphenols. As a result, it was postulated that 6-GG may alter the initial structure of MP, creating a compact, dense three-dimensional network between proteins. This would preserve the original MP structure while encouraging the development of a dense three-dimensional network between proteins, which would allow MP to retain more water. This observation was consistent with the WHC results of meatballs.

Meanwhile, as the cold storage time prolonged, the structure of Mb0 became gradually looser and rougher, with larger pores and unevenly distributed cracks. Similar changes were observed in the polyphenol treatment groups, but their structure was tighter with smaller pores and less uneven distribution of cracks compared to Mb0. On the other hand, Mb3 remained smooth with small pores and cracks until the 5th day of cold storage, which could be attributed to the good antioxidant properties of 6-GG, which effectively inhibited protein structural damage caused by protein oxidation. Fu et al. (2022) reported similar results. They found that shiitake polysaccharide (LNT) inhibited fat oxidation along with the release of nitrogenous substances. Furthermore, the presence of LNT facilitated the formation of the dense microstructure of goose meatballs during the entire storage period (3, 7, and 12 days). The quality of the goose meatballs was improved and lipid oxidation was slowed down by the introduction of LNT during cold storage. The microstructural results also correlated the findings of cooking loss, texture, LF-NMR, and MRI.

3.11. Correlation analysis

In Fig. 3, the addition of 6-GG was shown to be highly significantly positively correlated with gel strength, WHC, and various textural properties such as hardness, springiness, cohesiveness, chewiness, and resilience. It also showed correlations with PT_2, WHC, a*, and b* ($p <$

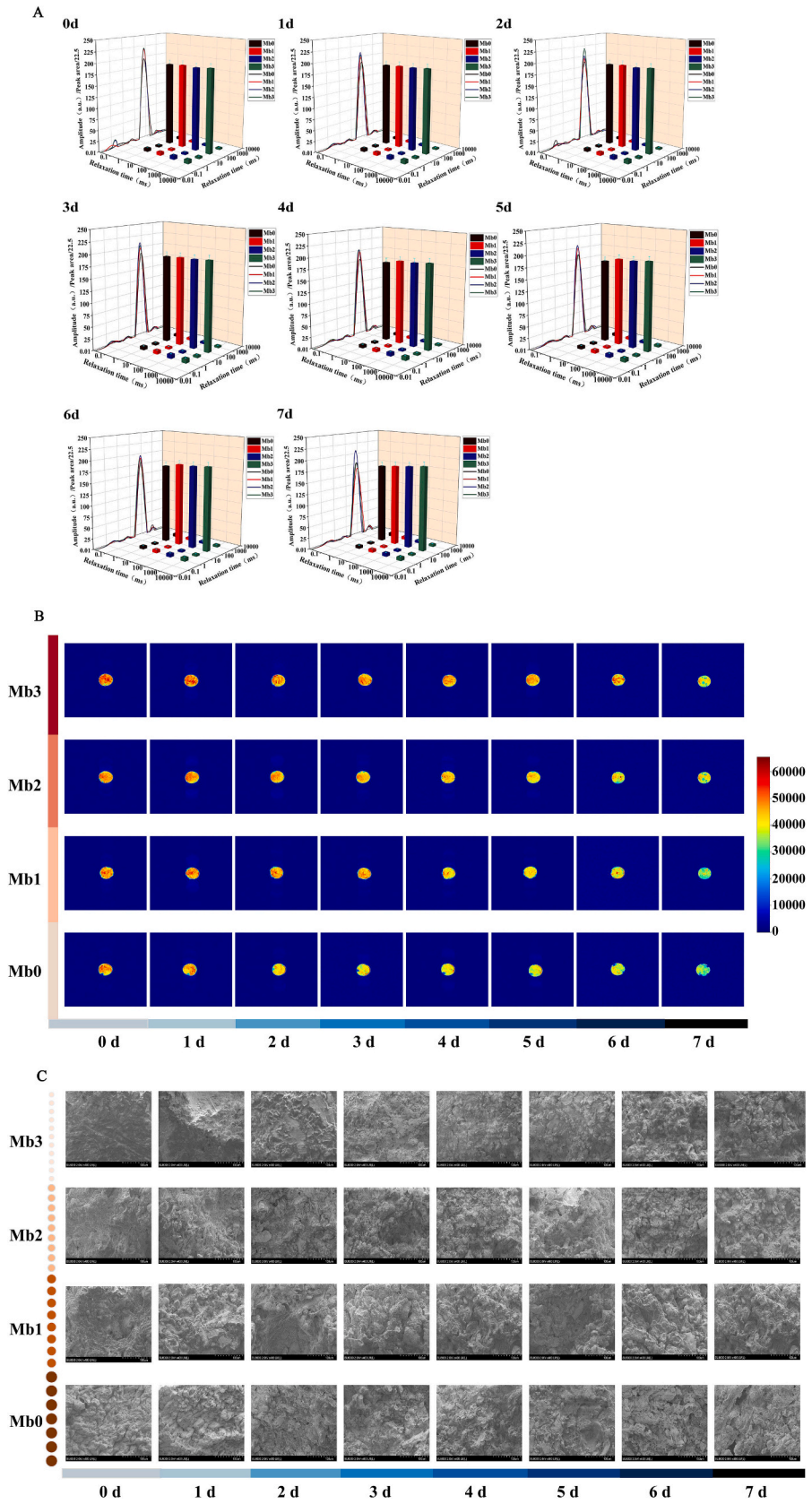


Fig. 2. Changes in the moisture distribution (A), moisture content (B), microstructural (C) of mutton meatballs during cold storage.
Note: The Mb0, Mb1, Mb2, and Mb3 treatment groups represent the addition of 0, 0.0088, 0.0176, and 0.0440% of 6-gingerol, respectively. 0d, 1d, 2d, 3d, 4d, 5d, 6d, and 7d represent days 0, 1, 2, 3, 4, 5, 6, and 7 of cold storage, respectively. In addition, the 0-60,000 is the range of values for the color scale (also known as the color bar or color scale), and here the larger the value, the higher the hydrogen proton density of the meatball.

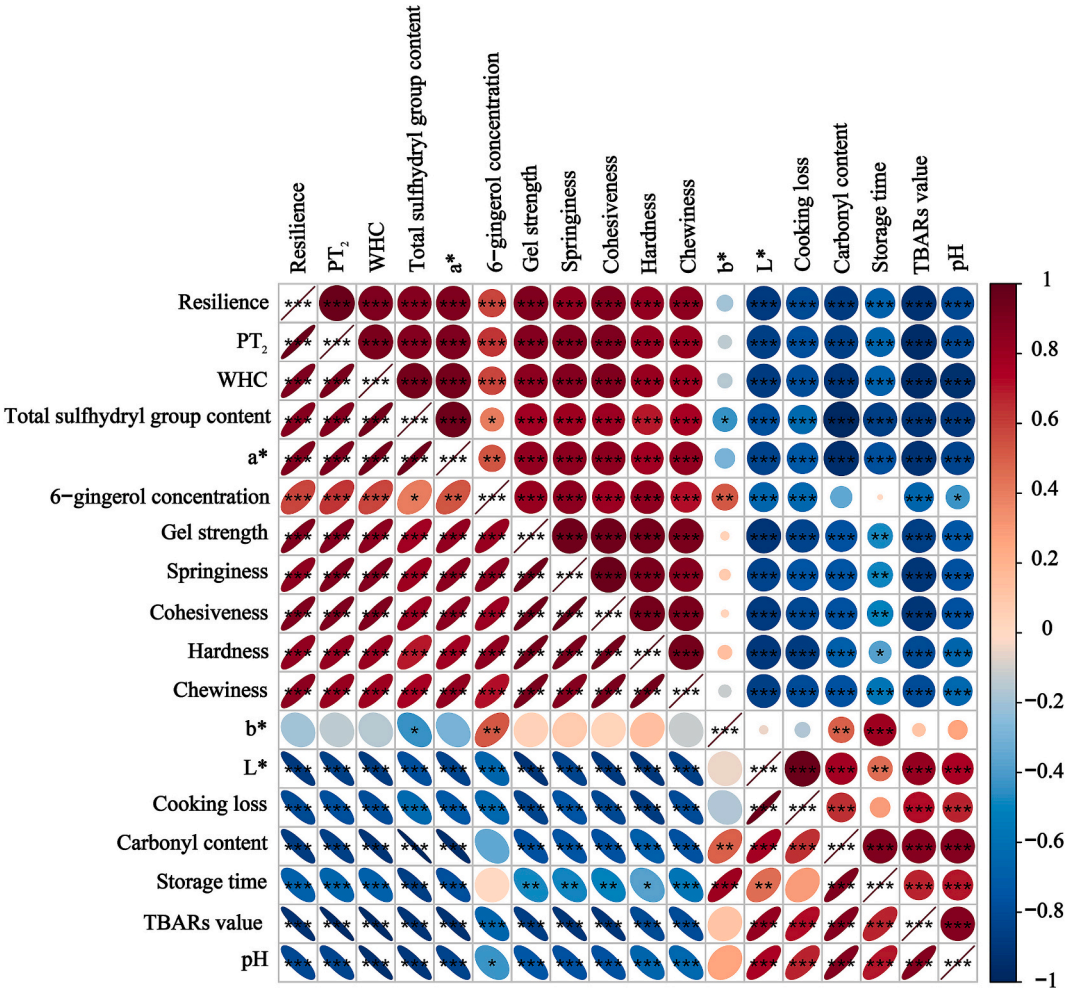


Fig. 3. Correlations between various indicators.
Note: PT_2 is the sum of the peak areas of bound, fixed, and free water in LF-NMR.

0.01). Also, it was significantly positively correlated with total protein sulfhydryl content ($p < 0.05$), highly significantly negatively correlated with TBARs, L*, and cooking loss ($p < 0.01$), and significantly negatively correlated with pH ($p < 0.05$). These findings suggested that the 6-GG treatment delayed muscle quality deterioration. Moreover, PT_2 (the sum of the peak areas of bound, fixed, and free water in LF-NMR) were highly significantly positively correlated ($p < 0.01$) with total protein sulfhydryl content, gel strength, a*, various textural properties, and WHC. Further, a strong and positive correlation ($p < 0.01$) was observed with protein carbonyl content, pH, TBAR value, L* value, and cooking loss. These findings suggest that moisture content and protein oxidation during cold storage have a significant impact on the quality of mutton meatballs (Cheng et al., 2020).

4. Conclusions

The current study shows that 6-GG treatment groups (Mb1, Mb2, and Mb3) exhibited a delay in protein and lipid oxidation along with the prolongation of the refrigerated shelf life of mutton meatballs, with improved texture and higher WHC and moisture content compared with the control group (Mb0). Moreover, the LF-NMR parameters and the quality parameters of the refrigerated mutton meatballs were found to be strongly correlated. Therefore, 6-GG could be added to meat products as a natural antioxidant to delay the oxidation process, in turn improving their quality. Furthermore, under the conditions of this experiment, 0.0176% of 6-GG addition was more appropriate based on the economic production and safe dosage. This informs the application of natural antioxidants in meat products.

CRediT authorship contribution statement

Jiamei Li: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Formal analysis, Data curation. **Lijie Wang:** Software, Investigation. **Hongyan Mu:** Conceptualization. **Geyi Ren:** Software, Investigation. **Mengyao Ge:** Investigation. **Juan Dong:** Supervision, Funding acquisition, Conceptualization. **Qingling Wang:** Resources, Conceptualization. **Jingtao Sun:** Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by the National Natural Science Foundation

of China (32360606), Shihezi University Innovation Development Special (CXFZ202206), and Corps Guiding Science and Technology Plan (2022ZD061). The Corps Talent Project in Xinjiang Production and Construction Corps.

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