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# Effects of lactic acid bacteria and yeast on mutton quality at different temperatures

Xinya JIAO<sup>1</sup>, Ying SHU<sup>1</sup>, Weili RAO<sup>1</sup>, Zhisheng ZHANG<sup>1\*</sup>, Shumei CHENG<sup>1\*</sup> 💿

# Abstract

At various temperatures, the effects of *Saccharomyces cerevisiae*, *Lactobacillus farciminis* S26, *Lactobacillus plantarum* S42 and *Pediococcus acidilactici* S53 on physical and chemical indicators as well as sensory quality of mutton were investigated. These characteristics are then used to build the shelf life prediction model. The results revealed that as the storage period rose, the moisture content and sensory quality of the mutton dropped, but the TBARS value increased. The theoretical shelf life of mutton at 4 °C, 25 °C, and 37 °C, according to the kinetic equation of TBARS value, is 59d, 19.7d, and 14.08d, respectively, suggesting that the theoretical value is in excellent agreement with the observed value. Finally, the combination of lactic acid bacteria and yeast has the potential to improve the shelf life of mutton. Simultaneously, low-temperature storage at 4 °C can improve mutton quality.

**Keywords:** Saccharomyces cerevisiae; Lactobacillus farciminis S26; Lactobacillus plantarum S42; Pediococcus acidilactici S53; mutton.

**Practical Application:** Saccharomyces cerevisiae, Lactobacillus farciminis S26, Lactobacillus plantarum S42 and Pediococcus acidilactici S53 co-fermentation of mutton is a very effective fresh-keeping method, which can make mutton have a unique flavor and prolong the storage time of mutton. It is of great significance to the preservation and processing of mutton.

# 1 Introduction

Lactic acid bacteria and yeast cause raw meat to undergo a sequence of biochemical and physical changes under natural or artificially controlled conditions, resulting in meat products with a distinct flavor, color, texture, and long shelf life (Asaduzzaman et al., 2020; Cruxen et al., 2019; Petrović et al., 2022; Skoufogianni et al., 2019). Famous fermented meat items in China include Zhejiang Jinhua ham, Hunan pickled pork, and Shanxi pickled mutton, among others (Zhao et al., 2011). Fermented fish is produced and consumed in many regions of the world, and it is an integral element of many cuisine traditions (Isola et al., 2022). Elsewhere, Turkish dry fermented sausage is a traditional and very popular meat product that is widely consumed in Turkey (Şimşek, 2022). To achieve the traditional fermentation of mutton, processors frequently utilize lactic acid bacteria, processing equipment, and the atmosphere (Holko et al., 2013; Klingberg et al., 2005). Lactic acid bacteria are crucial in the lives of natural microorganisms. Lactic acid bacteria are the principal groups of bacteria used in foods, as they have a historically long and secure use and are established as generally recognised as safe (Lucatto et al., 2020). They have the ability to ferment sugar into acidity, reduce pH, improve product texture and stability, limit the spread of food pathogens, and create fragrant chemicals (Yerlikaya et al., 2020). Debaryomyces hansenii on Italian sausage improves its quality and adds a delightful flavor. Yeast has high drug resistance, as well as acid, salt, and temperature tolerance (Balia et al., 2018; Suryaningsih et al., 2019).

At present, the shelf-life prediction of meat products is mainly done through dynamic models, among which the most widely used dynamic models are zero-order and first-order dynamic models. For example, Dalcanton et al. (2013) used firstorder kinetic model fitting combined with vacuum packaging to explore the growth and change laws of microorganisms in cooked pork products and predict their shelf life. Deyang Li et al. (2019) predicted the shelf life of hot air-dried and freeze-dried Penaeus vannamei by using the accelerated storage test and the Arrhenius equation.

This experiment studied the changes of various indexes after adding *Saccharomyces cerevisiae*, *Lactobacillus farciminis S26*, *Lactobacillus plantarum S42*, and *Pediococcus acidilactici S53* to mutton at a different temperature and analyzed the correlation of various indexes. At the same time, on this basis, the shelf life of mutton under different temperature conditions is predicted, which provides some theoretical guidance for quality monitoring in the process of production, storage, transportation, and sales.

# 2 Materials and methods

## 2.1 Bacterial strains and inoculum preparation

Dong traditional fermented sour meat from Hebei Agricultural University (Baoding, Hebei, China) is separated into *Lactobacillus farciminis S26*, *Lactobacillus plantarum S42*, and *Pediococcus acidilactici S53* (Man-man et al., 2020). 20% (v/v) glycerol was

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<sup>&</sup>lt;sup>1</sup>College of Food Science and Technology, Hebei Agricultural University, Baoding, China \*Corresponding author: zzs324@126.com, 516089669@qq.com

added to the MRS broth (Beijing AOBOX Biotechnology Co., Ltd., Beijing, China) of pure culture and stored at -80 °C.

After the activation of *Lactobacillus farciminis* S26, *Lactobacillus plantarum S42*, and *Pediococcus acidilactici S53*, Lactic acid bacteria were inoculated in MRS medium (AOBOX) at 37 °C for 24 hours, and *Saccharomyces cerevisiae* (Angel Yeast, China) was inoculated in YEPD medium (AOBOX) at 28 °C for 24 hours. After the completion of the culture, the bacterial solution was centrifuged at 6000 R / min for 10 min, the supernatant was discarded, the precipitation was washed with normal saline 3 times, and finally, sterile water was added for standby.

## 2.2 Preparation of mutton

Cut the mutton into 3cm  $\times$  3cm  $\times$  3 cm pieces with cold water. Then mix the meat with the following ingredients: monosodium glutamate 2 g/kg, sucrose 12 g/ kg, glucose 5 g/ kg, salt 20 g/kg, pepper 2.5 g/kg, dried orange peel 1 g/kg, ginger 5 g/kg, onion powder 5 g/kg, cloves 2 g/kg, cinnamon 3 g/kg, cumin 10g/kg, white wine 10g/kg, chili powder 10 g/kg, sodium nitrite 0.15 g/kg, sodium nitrate 0.5 g/kg, and Vc 0.4 g/kg, all provided by Beiguo supermarket (Baoding, Hebei, China). The mixed meat was pickled in a refrigerator at 4 °C for 48 hours. Add 1% Lactobacillus farciminis S26, Lactobacillus plantarum S42, Pediococcus acidilactici S53, and Saccharomyces cerevisiae (2:4:3:2) to the mixed meat. Then, 25 grams of pretreated meat pieces were put into a sterilization flask, sealed with plastic film, and kept in a 28 °C incubator for 20 hours. Finally, the mutton was packaged in a sterile bag and stored at 4 °C, 25 °C, and 37 °C, respectively.

## 2.3 Total bacterial count

The method for determining the total number of colonies adopts the national standard GB 4789.2-2016 determination of the total number of colonies in the microbiological examination of food. lg CFU/g is the unit of measurement for the outcome.

#### 2.4 Physicochemical composition

The PH of the samples was determined with a digital pH meter (PHS-25, Inesa instrument, Shanghai, China) in accordance with GB 5009.237-2016 (China). A homogeneous mixture of the samples was dried directly at  $100 \pm 5$  °C to constant weight according to GB 5009.3-2016 (China). to determine moisture. Cut a 1 cm thick meat sample, expose the cut surface to air for 30 minutes, and then use a color difference meter (WSC-2B Color Reader, Inesa, Shanghai, China) (Özer & Kilic, 2015) to determine the values of L\*, a\*, and B\*. According to Bourne (1966), texture measurements (TMS-Pro, Food Technology Corporation, Shanghai, China) were taken using a texture property. And each experiment was carried out at least three times.

#### 2.5 Sensory evaluation

The degree of difference and descriptive sensory analysis was performed at the Department of Food Sciences at the Agricultural University of Hebei Province by a group of ten, nonsmoker panelists (5 males and 5 females, between 20 and 30 years old) experienced in the sensory evaluation of foods. Sensory attributes—including color, flavor, texture, sourness, saltiness, and sweetness-were evaluated using a 7-point scale, where 1 = light color and low intensity of attributes and 7 = dark color and high intensity of attributes (Hongthong et al., 2020). Rinse with water and interval of 10 minutes when evaluating each product. Then, according to the evaluation results, after removing the highest and lowest scores, calculate the average value as the final score of the sample.

## 2.6 Helf life prediction

The Pearson correlation analysis method was used to analyze the indexes of mutton during storage in order to get the key indexes during storage, and then the kinetic model suitable for this experiment was determined by the determination coefficient of the regression equation. Arrhenius equation (Ratkowsky et al., 1982) (Equation 1) reflects the relationship between reaction rate and temperature.

$$\ln k = \frac{-E_a}{RT} + \ln k_0 \tag{1}$$

Where k is the reaction rate constant; Ea is the apparent activation energy, kJ/mol, T is the storage temperature, R is  $8.314 \text{ J/}(\text{mol}\cdot\text{k})$ , and k0 is the pre-exponential factor.

ln k and T<sup>-1</sup> were linear relationship by Equation 1, the straight slope to-Ea/R, ln k0 represents the intercept on the Y-axis. After obtaining the k at three different storage temperatures, Ea and  $k_0$  can be calculated by plotting ln k<sup>-1</sup> to T.

Combined with the kinetic model and the Arrhenius equation, the shelf life of mutton was obtained, and the prediction model by Equation 2.

$$SL = \frac{\ln \frac{B}{B_0}}{k_0 \exp\left(\frac{-E_a}{RT}\right)}$$
(2)

#### 2.7 Statistical analysis

The data was analyzed using SPSS 22.0 software, oneway ANOVA, and the Tukey HSD technique for multiple comparisons to determine the significance of the difference between the treatment groups, and mapping was done using Origin 8.5 software. The experiment was done three times and the data were expressed as mean standard deviation.

## 3 Results and discussion

## 3.1 Total bacterial count

In this experiment, mutton was taken as the test material, and samples were taken on days 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, and 30 (storage period). The results showed that the total number of bacteria in frozen mutton slices increased with the extension of storage time and with the increase of storage temperature, as shown in Figure 1a. Under the storage conditions of 25 °C and 37 °C, the total number of bacteria in mutton first increased, then decreased slightly and tended to be stable. This is because mutton does not completely consume all carbohydrates in the early stage of storage, and lactic acid bacteria and yeast

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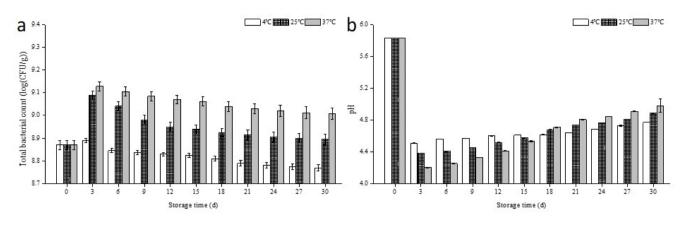


Figure 1. Changes in (a) total bacterial count and (b) pH value of mutton during processing.

continue to grow at an appropriate temperature. The pH value of mutton decreased with the extension of time, and the residual carbohydrate decreased gradually, which was not conducive to the continuous growth of lactic acid bacteria and yeast. This makes the total number of bacteria gradually decrease and then stabilize. The total number of bacteria did not change significantly under cold storage conditions (4°C). This is because the total number of bacteria is not suitable for growth and inhibits the growth of other bacteria during low temperatures. This makes the total number of bacteria change slowly.

## 3.2 pH measurement

The pH of meat products is an important indicator. The study's findings (Figure 1b) revealed a quick fall in pH values for all treatments, followed by a significantly higher. This is due to the fact that mutton has not yet been fully fermented and contains a high concentration of lactic acid bacteria at the end of the mutton fermentation. Because the optimum temperature for lactic acid bacteria was 37 °C, the pH of mutton held at 37 °C declined at a faster rate. And the pH of mutton held at 4 °C and 25 °C declines slowly. Protein and fat are degraded by lactic acid bacteria as storage duration and pH value increase, forming basic nitrogen compounds that gradually increase, especially at 37 °C.

#### 3.3 Instrumental colors

L\* value is an important index to describe the brightness of the sample. The higher the L\* value, the better the brightness of the mutton. a\* value represents the red of the sample, and b\* value represents the yellow of the sample.

It can be seen from Figure 2 that the pH was found to decrease due to lactic acid bacteria continuing to ferment at the initial stage of storage. And nitrite is easier to decompose to form a nitroso group, and bind to the myoglobin bringing meat color turn red and light. Therefore, the L\* values and the a\* values show an upward trend at the early stage of storage. The L\* values and the a\* values gradually decreased with the increase in the storage time. The higher the temperature, the greater the magnitude of the change of the L\* values and the a\* values. This is related to the progressive breakdown of protein

and the constant oxidation of fat, which eventually results in the blackening of meat during storage. It was also discovered that there was no clear pattern to the fluctuation in the b\* values, which overall indicated an increasing tendency.

#### 3.4 Moisture content

As shown in Figure 3, the pH value of mutton under varied storage settings decreases as storage time increases. The higher the temperature, the greater the decrease of water content. Due to the increase of pH value in the later storage period, the water holding capacity of meat becomes worse (Tornberg, 2005). The water loss at 4 °C is less than 25 °C and 37 °C.

# 3.5 TBARS

TBARS, as an indicator of fat oxidation, increased as expected throughout the processing (Figure 4). The storage temperature had a significant effect on the fermentation value of mutton. The results showed that TBARS of mutton was 0.15 mg/kg at 0-day storage. TBARS of mutton at different storage temperatures increased slowly before 15 days. When stored for 15 days, the TBARS of mutton was about 0.49 mg / kg at 4 °C, 0.53 mg / kg at 25 °C and 0.90 mg / kg at 37 °C.

After that, with the extension of storage time, the value of mutton can grow in different ranges at different storage temperatures. Among them, the lipase of mutton is easy to activate at 37 °C, which is conducive to the growth and reproduction of bacteria. Under the joint action of enzymes and microorganisms, it accelerates the oxidative rancidity of meat products. In short, the higher the storage temperature, the faster the TBARS value increases, which is more likely to lead to meat corruption.

#### 3.6 Texture profile analysis and sensory evaluation

Figure 5 shows that the hardness, chewiness, and elasticity of mutton changed significantly with the extension of storage time (P < 0.05). The hardness and chewiness of mutton increased on days 0 and 3 and then decreased gradually. In addition, the overall elasticity showed a downward trend. The increased hardness is mainly because of the acid-induced denaturation and gelation of

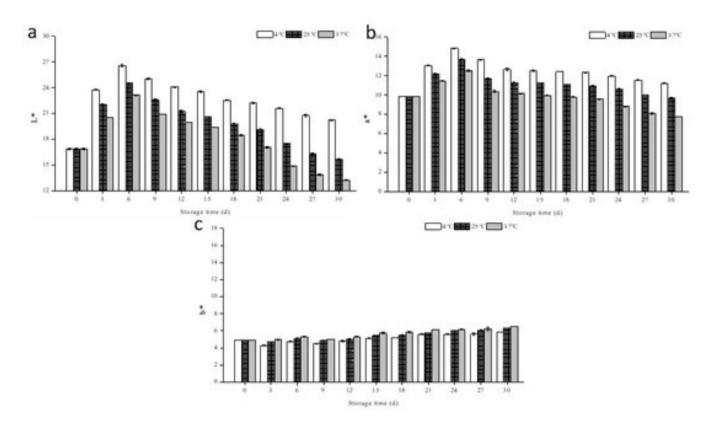


Figure 2. Changes in (a) L\*, (b) a\*, and (c) b\* values of mutton during processing.

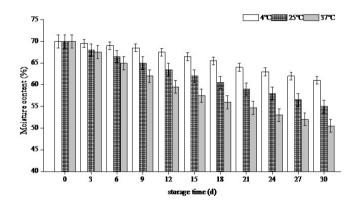


Figure 3. Changes in moisture content of mutton during processing.

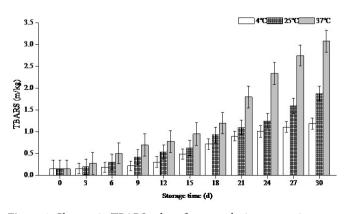


Figure 4. Changes in TBARS value of mutton during processing.

meat proteins. Lactic acid bacteria no longer continue to lower acidity as storage time increases, and the protein in mutton is continuously destroyed by microbes and enzymes, causing the texture of mutton to deteriorate after fermentation. At 4 °C, the texture change trend of mutton changed little compared with 25 °C and 37 °C. At low temperatures, the water loss rate of lactic acid bacteria is lowered, and the activities of lactic acid bacteria and enzymes are suppressed.

Figure 6 illustrates the sensory evaluation results of the mutton during processing, showing that the colors of the samples darkened, which was consistent with the instrumental determination of the lower L\* values. Many factors influence the development of the unique fermented flavors, including the product formulation, starter cultures, and processing conditions. Through the actions of microbial and endogenous meat enzymes, the breakdown of carbohydrates, lipids, and proteins, particularly those generating volatile components, might have contributed to the fermented flavors of the samples. Under the storage condition of 4 °C, the sensory evaluation value of mutton decreased slowly. Other characteristics, including salinity and sweetness, did not change significantly. Therefore, it is suggested to store mutton at low temperatures, which is beneficial to prolong the shelf life and improve the sensory quality.

#### 3.7 Chemical reaction kinetics model analysis

The selection of the key indicated factors. The Pearson's correlation coefficient between the TBARS values and the sensory evaluation is 0.954, 0.960, and 0.986 under storage at



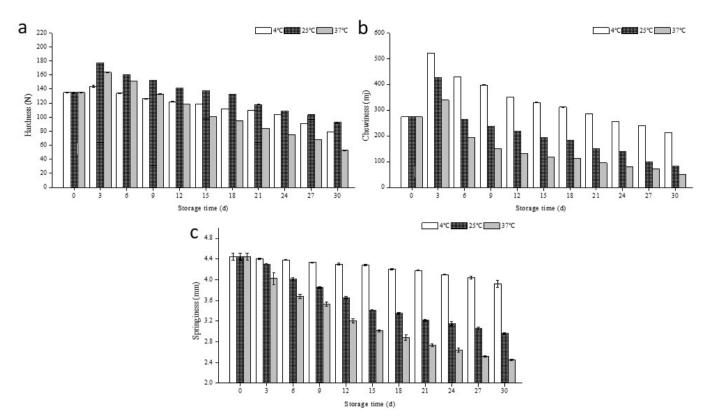


Figure 5. Changes in texture profile analysis including (a) hardness, (b) chewiness and (c) springiness of mutton during processing.

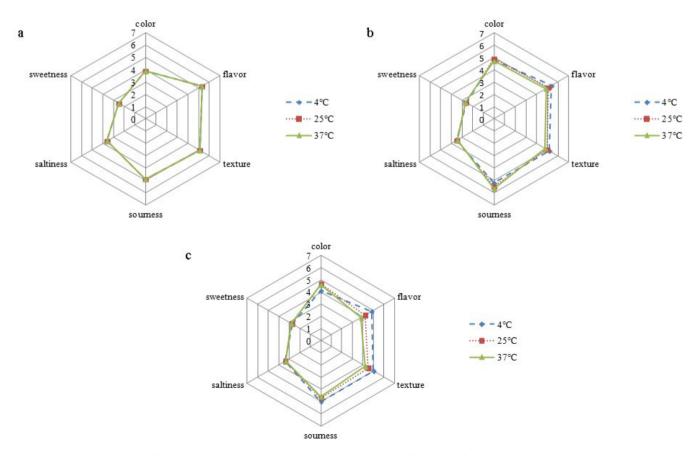


Figure 6. Sensory evaluation of mutton during processing and storage at (a) day 0, (b) day 9 and (c) day 12.

4 °C, 25 °C, and 37 °C. Thus, we took the PH values as the key indicated factors of the quality change of mutton and the shelf live dynamics predicted model. The results showed that when the concentration of mutton fermentation broth was greater than 1.2 mg / 100 g, the mutton could not be eaten and the shelf life reached the endpoint.

The regression equation of TBARS values of mutton chang with storage time under different storage temperatures. The TBARS values of mutton under different storage temperatures kept increasing with storage time. From Table 1 and Figure 7, the TBARS values are linear with storage time, and any one of the R<sup>2</sup> of the regression equation was greater than 0.9, which indicates high confidence in the forecast models. Under three different storage temperatures, the change rates of TBARS value (k) are as follows:  $k_4 = 0.0299$ ,  $k_{25} = 0.1165$  and  $k_{37} = 0.5803$  ( $k_4$ ,  $k_{25}$  and  $k_{37}$  are denoted reaction rate at 4 °C, 25 °C and 37 °C, respectively).

 Table 1. Regression equations of TBARS values of mutton at different storage temperatures as a function of storage time.

storage temperatures (°C)	regression equation	R2	k	Ink
4	y=0.0474x-1.942	0.9891	0.0474	-3.05
25	y=0.1037x-2.1491	0.9747	0.1037	-2.27
37	y=0.2005x-1.8819	0.9867	0.2005	-1.61

The TBARS values of mutton changing Arrhenius equation. From Figure 8, the linear regression equation was y = -3166.6x + 8.5005 ( $R^2 = 0.9677$ ), by Equation 3:

$$\ln k = -\frac{E_a}{RT} + \ln k_0 \tag{3}$$

The calculated activation energy of changes in pH is Ea = 25.846 kJ/mol and pre-exponential factor is  $k_0 = 3.52 \times 10^3$ . The following Arrhenius equation for TBARS values was obtained (Equation 4):

$$k = 3.52 \times 10^3 \times \exp\left(\frac{-25.846 \times 10^3}{\text{RT}}\right)$$
(4)

Predictive modeling of the shelf life of mutton. Substituting Ea of 25.8846 kJ/mol and  $k_0$  of  $3.52 \times 10^3$  into the formula, lnA = lnA<sub>0</sub> + kt, was deformation of first-order kinetic equation A = A<sub>0</sub> exp (kt). we obtained first-order kinetic equation of shelf life of mutton (Equation 5):

$$\ln A = \ln A_0 + 3.52 \times 10^3 \times \exp\left(\frac{-3.11 \times 103}{273 + a}\right) \times t$$
(5)

The predictive modeling of the shelf life obtained after deformation (Equation 6):

$$=\frac{\ln A - \ln A_0}{3.52 \times 10^3 \times \exp\left(\frac{-3.11 \times 10^3}{273 + a}\right)}$$
(6)

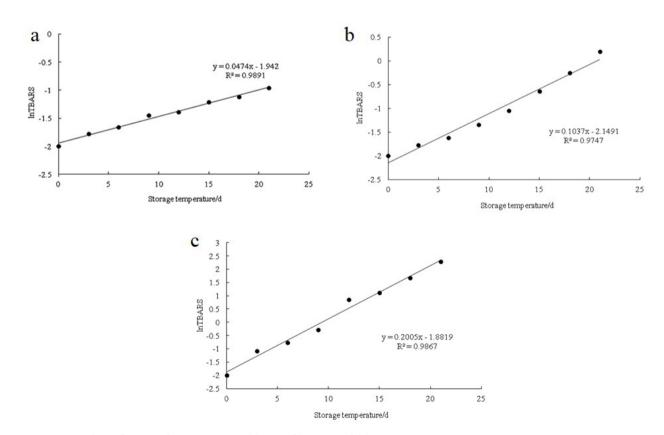


Figure 7. TBARS values of mutton during storage at (a) 4 °C, (b) 25 °C and (c) 37 °C.

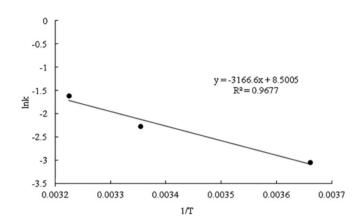


Figure 8. Arrhenius curve of TBARS value of mutton.

**Table 2**. Shelf life verification of mutton.

Storage temperature (°C)	predicting shelf-life (d)	actual shelf-life (d)	relative error (%)
4	59	62	4.83%
25	19.7	18	9.44%
37	14.08	15	6.13%

Where A is the TBARS values (mg/100),  $A_0$  is the initial value of TBARS, a is storage temperature (°C), and t is storage time (d). Eventually, the initial value of TBARS determined as 0.15 mg/100g, storage end point TBARS is 1.2 mg/100 g. If mutton were stored at 4-40 °C, predictive modeling of the shelf life can be inferred by shelf life.

The shelf life of prediction model and prediction of shelf life. From Table 2, relative errors between the predictive value and the measured value of that Arrhenius equation of mutton were 4.83%, 9.44%, and 6.13% during storage at 4 °C, 25 °C, and 37 °C. The relative errors between the predicted and the measured values are within 10%, have a high fitting degree. Therefore, this method can accurately predict the quality change and shelf life of mutton during storage.

# **4** Conclusions

Under different storage temperatures, the moisture content and sensory quality of mutton decreased gradually with the extension of storage time. With the extension of storage time, TBARS value increased gradually, and the rising rate from fast to slow was 37 °C > 25 °C > 4 °C. Pearson correlation coefficient between sensory evaluation and TBARS values were greater than 0.9, so the TBARS values were selected as the key factor of the shelf life prediction model.

The Eq. (2) was combined to build the shelf life predictive model that with respect to rate constants rate of change (k) of TBARS values is associated with storage temperature (T) of TBARS values, the obtained shelf life prediction model is Eq. (6).

In the prediction model, the activation energy Ea is 25.8846 kJ/mol, and pre-exponential factor  $k_0$  is  $3.52 \times 10^3$ . Shelf-life predictive

values of mutton at different storage temperatures (4 °C, 25 °C, and 37 °C) to 59 d, 19.7 d, and 14.08 d, respectively, which is consistent with the measured values (62 d, 18 d, and 15 d).

# **Conflict of interest**

The authors declare no potential conflicts of interest.

# Data availability

The data generated or analyzed during this study are in cluded within this article. The data used can be acquired from the first author (516089669@qq.com) upon request.

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