

# Research On The Physicochemical Indicators During Fermentation Of Fermented Bean Curd

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## Abstract

*Fermented bean curd, a traditional Chinese fermented soybean product, boasts promising market prospects. Presently, as consumer demand for low-salt diets continues to grow, domestic and international scholars are developing and researching low-salt fermented bean curd. However, reducing the salt content may lead to changes in the components of fermented bean curd, which is not conducive to its preservation. This paper aims to study the changes in pH, acidity, and amino nitrogen of low-salt fermented bean curd at different temperatures during fermentation. Considering the risk of spoilage during transportation, this paper adopts the method of freeze-thaw treatment to study its effect on the pH, acidity, and amino nitrogen of fermented bean curd. The experiments were conducted on natural fermentation of low-salt fermented bean curd at three temperatures (20°C, 24°C, and 28°C), while simultaneously performing -80°C freeze-thaw treatment on the fermented bean curd. The results show that the pH, acidity, and amino nitrogen of low-salt fermented bean curd at different temperatures meet national standards; the pH of low-salt fermented bean curd shows a trend of first increasing and then decreasing before and after freeze-thaw treatment, while the acidity and amino nitrogen content gradually increase, and the results all meet national standards. Freeze-thaw treatment has little effect on its physicochemical parameters.*

**Keywords:** Low-salt fermented bean curd; Freeze-thaw treatment; Physicochemical indicators

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## I. Introduction

Fermented bean curd, a product derived from soybeans, stands as a representative of traditional Chinese fermented soy products. During fermentation, fermented bean curd produces unique flavor and nutritional characteristics. This is because its fermentation process involves complex biochemical conversion processes of microbial communities <sup>[1]</sup>. In production practice, fermented bean curd fermentation is divided into <sup>[2]</sup> two stages: pre-fermentation and post-fermentation. fermented bean curd relies on *Muco*, *Rhizopus* and other filamentous fungi <sup>[3]</sup> for protein hydrolysis and lipolytic action to degrade soybean matrix into small molecular peptides, free amino acids, and fatty acids, and this will make it easier to digest and increase the bioavailability of protein <sup>[4]</sup>. At the same time, it will also involve secondary metabolic pathways such as the Maillard reaction. The reactions promote the formation of flavor compounds in fermented bean curd <sup>[5]</sup> and ultimately forming a sensory characteristic that integrates “fresh, fragrant, mellow, glutinous”. The formation of flavor compounds in fermented bean curd is also related to the synergistic action of various enzymes <sup>[6]</sup>. Additionally,

fermented bean curd contains bioactive components such as flavonoids and peptides. These compounds demonstrate nutritional functionalities including antioxidant activity and cholesterol-lowering effects in humans [7].

This experiment studied the fermentation of fermented bean curd under low salinity conditions, but compared to high-salt fermented bean curd, low-salt conditions are more prone to spoilage and deterioration [8]. [9]. For this reason, the method of cold chain transportation must be adopted during transportation [10]. In this experiment, the fermented bean curd is rapidly frozen at -80°C, then simulate transportation at -18°C, and finally thaw and study. This freeze-thaw process may cause changes in the physicochemical indicators such as pH, acidity and others of the fermented bean curd. Therefore, this experiment analyzes the physicochemical indicators of fermented bean curd before and after freeze-thaw. The main purpose of this study is to study the physicochemical characteristics of low-salt fermented bean curd at different temperatures, and to study the use of cold chain transportation on the physicochemical indicators of this traditional fermented bean curd. The physicochemical components of food can directly reflect the properties and state of the food and its condition. The changes in the physicochemical components of fermented bean curd during fermentation can also indirectly indicate the fermentation and ripening status of fermented bean curd [11]. Through the research in this paper, we aim to provide a theoretical basis for the storage and transportation of low-salt traditional fermented bean curd.

## **II. Materials And Methods**

### **Experimental Materials**

Experimental fermented bean curd (Shicun Convenience Store Shanzi fermented bean curd), Nongfu Spring water, pepper corns and other spices (Mei yuan Restaurant of Shandong University of Technology). Analytical pure sodium hydroxide (China National Pharmaceutical Group Reagent Company). First, fresh fermented bean curd is cut into 3 cm × 3 cm × 2 cm size, the prepared fermented bean curd is placed in a sterilized container and arranged at intervals of 2cm in the container. Then, the container is placed in constant temperature incubator and keeping relative humidity 95%;fermented bean curd is fermented naturally at 20°C, 24°C and 28°C [12].This stage is the preliminary fermentation stage, where mold growth begins after about 3 days of fermentation, and the fermentation period is 7 days. Then the fermented bean curd is seasoned according to the commercial fermented bean curd recipe after marinating and then undergoes post-fermentation.

### **Experimental Method**

Mix fresh scallion sections, chopped scallions, pepper corn skins and other aromatic spices in the proportion of commercial soup base and place them in a bag, Li et al. [13] researched and the results indicate that aromatic spices not only serve a flavoring purpose but can also reduce the content of biogenic amines. Boil Nongfu Spring water and pour it into the container holding the bag until fully submerged, then let it cool and set aside. Once the braising liquid has naturally cooled to room temperature, add edible salt and dissolve it thoroughly for seasoning [13]. Measure 1000 g of fermented bean curd blocks, immerse them in the seasoned braising liquid containing high-proof liquor, and seal them in a constant-temperature environment for 24h. After soaking, fermented bean curd is mixed in an appropriate amount of MSG and transferred to a refrigeration device (4°C) for 24-48h until the flavor is fully developed, then it is ready for use.

### **Freeze-thaw treatment of Fermented Bean Curd**

During the fermentation process, the fermented bean curd is rapidly frozen at -80°C. Wang Mengze et al. [14] studied the effects of freeze-thaw treatment on the storage of wolfberry, and the results showed that freeze-thaw treatment can improve storage stability. After rapid freezing, it is stored at -18°C to allow ice

crystals to uniformly rearrange. Then, it is placed at room temperature in air to thaw to room temperature. After thawing, the fermented bean curd is labeled for data recording.

### **Data processing**

For each fermented bean curd sample under analysis, three independent replicate samples were prepared and designated as A, B, and C. The AVERAGE and STDEV functions were employed to calculate the mean values and standard deviations of the triplicate measurements, respectively. Experimental data were processed using Origin 2024 and Excel 2021, with subsequent generation of graphical representations.

## **III. Determination Of Physicochemical Indicators Of Fermented Bean Curd**

### **Determination of pH in Fermented Bean Curd**

First of all, take an appropriate amount of sample for homogenization treatment. Weigh 10.0g of the homogenized fermented bean curd sample into an Erlenmeyer flask. Add 50 mL of freshly boiled and cooled water, and mix thoroughly. Subsequently, filter the solution through rapid filter paper to collect the filtrate. Take 10 mL of the sample into a small beaker, and use a calibrated pH meter to measure the pH value <sup>[15]</sup>.

### **Total acid determination <sup>[16]</sup>**

Accurately weigh 25g (to the nearest 0.001g) of the homogenized sample into a 150 mL beaker. Subsequently, add 50 mL of carbon dioxide-free water preheated to 60°C to the beaker. Heat the mixture to boiling, followed by cooling to ambient temperature. Transfer the cooled mixture to a 200 mL volumetric flask for dilution, then filter the diluted sample through filter paper and collect the filtrate. Take 10 mL of the above filtrate into a beaker and add 50 mL of sterile water. Connect the pH meter to the power supply and calibrate it with a buffer solution, then place the beaker on a magnetic stirrer and stir at a speed of 300 r/min. Titrate the pH to 8.2 with a 0.05 mol/L (as specified by the national standard for lower acidity) sodium hydroxide solution. Continuously record the volume of the standard NaOH consumed and calculate the content of total acid in the fermented bean curd.

Simultaneously take 10 mL of sterile water as a reagent blank test and record the volume of standard solution consumed.

Calculation of total acid content in the sample:

$$x = \frac{(V_1 - V_2) \times c \times 0.090 \times 20}{m} \times 100 \quad \text{Equation 1}$$

c: Actual concentration of the sodium hydroxide standard solution (mol/L);

V<sub>1</sub>: Volume of NaOH solution consumed in the blank test (mL);

V<sub>2</sub>: Volume of NaOH solution consumed in the blank test (mL);

m: The numerical value of the mass of the sample, in units of grams (g);

X: Total acid content in the sample, unit of grams per hundred grams (g/100g);

20: Dilution factor of the test solution;

0.090: Mass of lactic acid equivalent to 1.00 mL of standard sodium hydroxide titrant solution, in units of grams (g).

### **Determination of amino nitrogen <sup>[17], [18]</sup>**

The experiment used the colorimetric method to determine the free amino nitrogen, with the method referenced from GB 5009.235-2016, and the specific experimental steps are as follows:

#### Standard curve preparation

Aliquot 0 mL, 0.05 mL, 0.1 mL, 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, and 1.0 mL of ammonia nitrogen standard solution into 10 mL colorimetric tubes. To each 10 mL colorimetric tube, add 4 mL of acetic acid-sodium acetate buffer solution (pH = 4.8) and 4 mL of chromogenic reagent, respectively, and used water to diluted the sample to the mark, then homogenized the mixture. Subsequently, transferred it to a 100°C water bath for 15 minutes. After heating, cool the sample to ambient temperature and transfer it into a 1 cm cuvette. Using the blank solution as reference, zero the spectrophotometer. Measure the absorbance of the standard curve solutions at 400 nm wavelength. Plot the data and calculate the linear regression equation.

#### Sample Determination

Take 30g of fermented bean curd sample and homogenize it in a homogenization bag. Weigh 5g ( $m_1$ ) homogenized sample into a 150 mL beaker and add 40 mL of newly boiled sterile water which cooled to room temperature. Transfer the solution to a 100 mL ( $V_2$ ) volumetric flask for dilution to volume and mix, then filter through rapid filter paper to collect the filtrate. Take 0.5 mL ( $V_1$ ) filtrate into a 10 mL colorimetric tube, add 4 mL acetic acid-sodium acetate buffer solution (pH = 4.8) and 4 mL color-developing agent. Dilute the reagent with water to the mark and mix thoroughly. Next, heat it in a 100°C water bath for 15 minutes, then remove it and cool to ambient temperature. Transfer the solution to a 1 cm cuvette, use the blank as the reference, and measure the absorbance of the sample solution at a wavelength of 400 nm. Simultaneously take an equal amount of sample and perform a blank test under the same conditions. The sample absorbance is corrected by subtracting the blank absorbance, after which the corrected absorbance is substituted into the linear regression equation to determine the free amino nitrogen content in the sample(m).

$$x = \frac{m}{m_1 \times 1000 \times 1000 \times \frac{V_1}{V_2}} \times 100 \quad \text{Equation 2}$$

X: Free amino nitrogen content in the tested sample (g/100g);

M: Mass of nitrogen in the tested sample determination solution ( $\mu\text{g}$ );

$V_1$ : Volume of the sample solution used for determination (mL);

$V_2$ : Volume of dilution in sample pretreatment (mL);

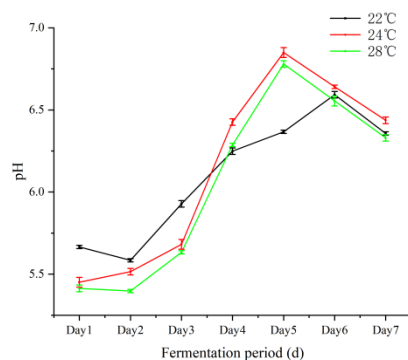
$m_1$ : Mass of the sample taken, in grams (g);

100, 1000: Unit conversion coefficient.

## IV. Indicator Characteristic Analysis

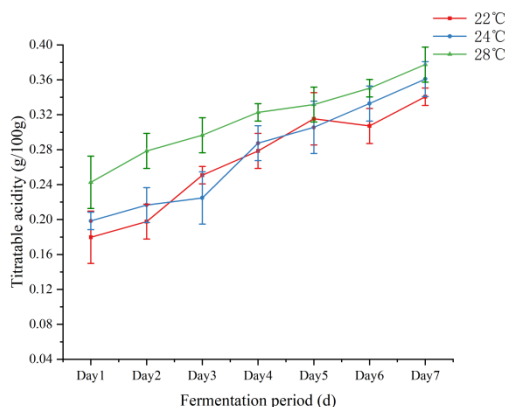
### Analysis of physicochemical indicators during Fermented Bean Curd fermentation at different temperatures

In the process of low-salt fermented bean curd fermentation, important physicochemical indicators were detected. Monitoring of key physicochemical indicators is not only the core link to ensure product quality but also an important basis for judging whether it meets the national industry standards (SB/T 10170). Detected indicators mainly include pH value, total acid content, and the content of amino nitrogen. According to the edible standard SB/T 10170, the content of amino nitrogen in fermented bean curd should be  $\geq 0.35$  g/100g, and the total acid content should be  $\leq 1.30$  g/100g [19].



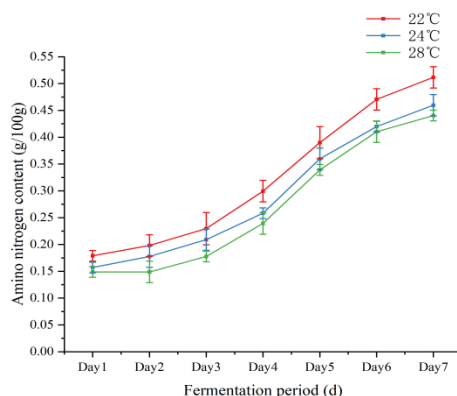
**Figure 1:** Variation in pH during Fermented Bean Curd fermentation at varying temperatures

The pH change curve in Figure 1 shows that under three temperature conditions (20°C, 24°C, 28°C), The pH value of the fermented bean curd remained within the safe range of 5.4-6.7 during the first 7 days. The result exhibits a trend of first increasing and then decreasing, which is consistent with the research findings of Zhou Ying et al. [20]. This change may be due to the protease secreted by the mold during the initial fermentation stage hydrolyzing soy protein into alkaline amino acids, leading to an increase in pH. Subsequently, the proliferation of acid-producing bacteria such as lactic acid bacteria caused the accumulation of organic acids, resulting in a slight decrease in pH [21]. If the measured pH value exceeds 8, it indicates that the food has already spoiled and is no longer edible, which making pH an important physicochemical indicator. Under the three temperature conditions of 20°C, 24°C, 28°C, the fermented bean curd samples all meet the standards.



**Figure 2:** Variation in acidity during Fermented Bean Curd fermentation at varying temperatures

From the data in Figure 2, it can be observed that the acidity of the fermented bean curd gradually increases, with a slower rate of increase in the later stage. The fermented bean curd at all different fermentation temperatures meets the acidity standard for fermented bean curd, with the 28°C condition showing the fastest change and the highest acidity compared to the other two temperature conditions. The acidity of the fermented bean curd is formed by free amino acids and organic acids produced during fermentation [22].



**Figure 3:** Variation in amino nitrogen content of Fermented Bean Curd fermented at varying temperatures

Figure 3 intuitively reflects the protein degradation efficiency of the free amino nitrogen curve, and the free amino nitrogen content can also be used as a basis for determining the ripeness of fermented bean curd [22]. From the data in the figure, it can be concluded that the content of free amino nitrogen gradually increases during fermentation. Zhou Ying et al. studied and the results [20] showed that during the fermentation process of fermented bean curd, the content of free amino nitrogen gradually increases, which is relatively consistent with the results of this study. The gradual increase in the content of free amino nitrogen indicates that proteins are continuously decomposed and the content of amino acids gradually increases. Among them, the fermented bean curd at 20°C reached the national standard of 0.35 g/100g first on the fifth day, and was higher than the other two fermentation temperatures, which may be because some microorganisms have a stronger ability to decompose proteins during low-temperature fermentation.

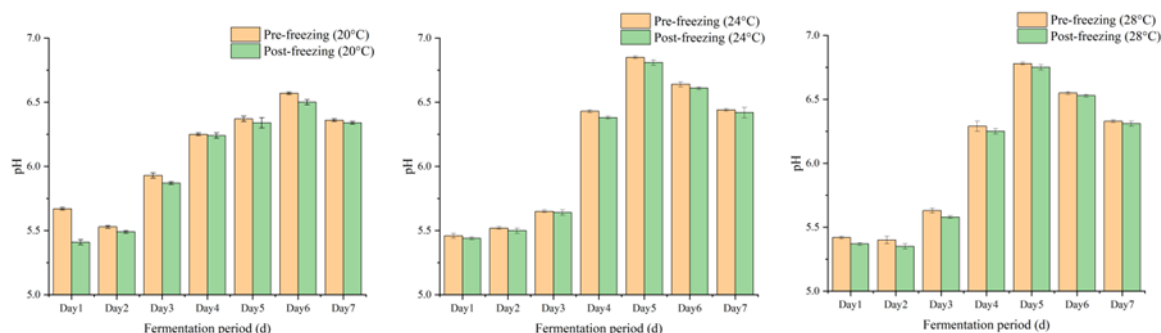
#### Analysis of physicochemical indicators of Fermented Bean Curd before and after freeze-thaw treatment

After rapid freezing at -80°C, frozen storage at -18°C, and room temperature recovery, samples were immediately taken in parallel for the detection of various physicochemical indicators of the freeze-thaw treated fermented bean curd, the results are as follows:

**Table 1:** Changes in the pH value of Fermented Bean Curd before and after freezing and thawing

Temperature (°C)	Fermentation period (d)						
	Day1	Day2	Day3	Day4	Day5	Day6	Day7
Pre-freezing (20°C)	5.67±0.01	5.53±0.01	5.93±0.02	6.25±0.01	6.37±0.02	6.57±0.01	6.36±0.01
Post-freezing (20°C)	5.41±0.02	5.49±0.01	5.87±0.01	6.24±0.02	6.34±0.04	6.5±0.02	6.34±0.01
Pre-freezing (24°C)	5.46±0.02	5.52±0.01	5.65±0.01	6.43±0.01	6.85±0.01	6.64±0.02	6.44±0.01
Post-freezing (24°C)	5.44±0.01	5.5±0.02	5.64±0.02	6.38±0.01	6.81±0.02	6.61±0.01	6.42±0.04
Pre-freezing (28°C)	5.42±0.01	5.4±0.03	5.63±0.02	6.29±0.04	6.78±0.01	6.55±0.01	6.33±0.01
Post-freezing	5.37±0.01	5.35±0.02	5.58±0.01	6.25±0.02	6.75±0.02	6.53±0.01	6.31±0.02

(28°C)							
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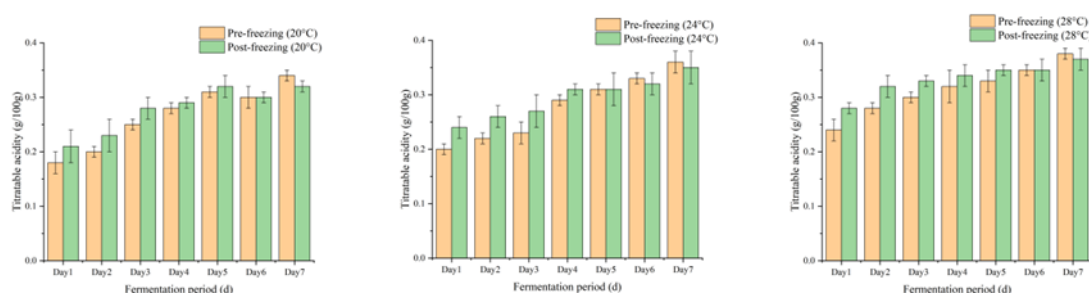


**Figure 4:** PH variation in Fermented Bean Curd before and after freeze-thaw treatment

Integrating the data presented in Table 1 and Figure 4, it can be inferred that the pH value of fermented bean curd declines after freeze-thawing. This phenomenon might be attributed to temperature fluctuations during the air thawing process, causing repeated melting and freezing of ice crystals, which enlarged the interstitial spaces in the fermented bean curd. This will increase its water content, and provided favorable conditions for the recovery of metabolic activity of cold-tolerant acid-producing microorganisms (such as lactic acid bacteria). The thawing process may lead to an increase in acidity <sup>[23]</sup> <sup>[24]</sup>. However, the data from Table 1 and Figure 4 indicate that the fermented bean curd samples at three temperatures all meet the standards after freeze-thawing.

**Table 2:** Changes in acidity during the fermentation of Fermented Bean Curd before and after freezing and thawing

Temperature (°C)	Fermentation period (d)						
	Day1	Day2	Day3	Day4	Day5	Day6	Day7
Pre-freezing (20°C)	0.18±0.02	0.20±0.01	0.25±0.01	0.28±0.01	0.31±0.01	0.30±0.02	0.34±0.01
Post-freezing (20°C)	0.21±0.03	0.23±0.03	0.28±0.02	0.29±0.01	0.32±0.02	0.30±0.01	0.32±0.01
Pre-freezing (24°C)	0.20±0.01	0.22±0.01	0.23±0.02	0.29±0.01	0.31±0.01	0.33±0.01	0.36±0.02
Post-freezing (24°C)	0.24±0.02	0.26±0.02	0.27±0.03	0.31±0.01	0.31±0.03	0.32±0.02	0.35±0.03
Pre-freezing (28°C)	0.24±0.02	0.28±0.01	0.30±0.01	0.32±0.03	0.33±0.02	0.35±0.01	0.38±0.01
Post-freezing (28°C)	0.28±0.01	0.32±0.02	0.33±0.01	0.34±0.02	0.35±0.01	0.35±0.02	0.37±0.02

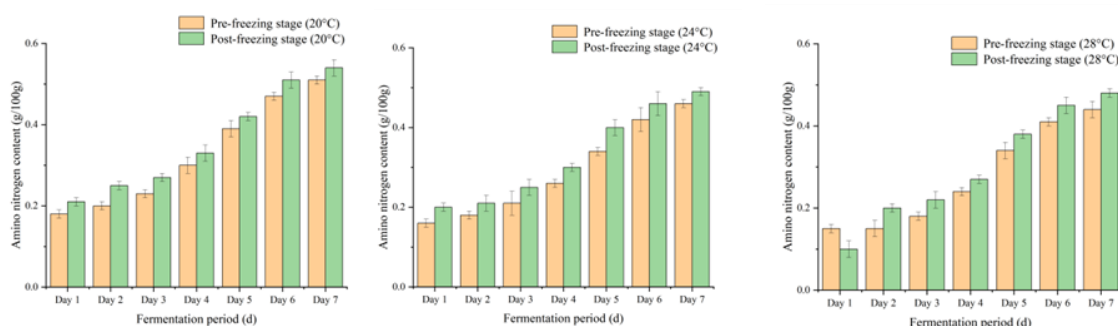


**Figure 5:** Titratable acidity variation in Fermented Bean Curd before and after freeze-thaw treatment

Analysis of the data in Table 2 and Figure 5 shows that the acidity of fermented bean curd after freeze-thaw treatment still exhibits a gradually increasing trend, but the acidity is higher than before freeze-thaw. At the three temperatures (20°C, 24°C, 28°C), the total acidity content of all samples increased by 0.20 – 0.30 g/100g compared to the control without freeze-thaw, which is consistent with the changes in pH value. Moreover, all samples did not exceed the upper limit specified in SB/T 10170 ( $\leq 1.30$  g/100g).

**Table 3:** Changes in amino nitrogen during the fermentation process of Fermented Bean Curd before and after freeze-thaw treatment

Temperature (°C)	Fermentation period (d)						
	Day1	Day2	Day3	Day4	Day5	Day6	Day7
Pre-freezing (20°C)	0.18±0.01	0.20±0.01	0.23±0.01	0.30±0.02	0.39±0.02	0.47±0.01	0.51±0.01
Post-freezing (20°C)	0.21±0.01	0.25±0.01	0.27±0.01	0.33±0.02	0.42±0.01	0.51±0.02	0.54±0.02
Pre-freezing (24°C)	0.16±0.01	0.18±0.01	0.21±0.03	0.26±0.01	0.34±0.01	0.42±0.03	0.46±0.01
Post-freezing (24°C)	0.20±0.01	0.21±0.02	0.25±0.02	0.30±0.01	0.40±0.02	0.46±0.03	0.49±0.01
Pre-freezing (28°C)	0.15±0.01	0.15±0.02	0.18±0.01	0.24±0.01	0.34±0.02	0.41±0.01	0.44±0.02
Post-freezing (28°C)	0.10±0.02	0.20±0.01	0.22±0.02	0.27±0.01	0.38±0.01	0.45±0.02	0.48±0.01



**Figure 6:** Amino nitrogen variation in Fermented Bean Curd before and after freeze-thaw treatment



According to Table 3 and combining the data from Figure 6, it can be concluded that the trend of change in the content of amino nitrogen in fermented bean curd after thawing is consistent with that before thawing, but the values have increased. The overall content of amino nitrogen in fermented bean curd after thawing increased by about 0.03 – 0.05 g/100g (as amino acid nitrogen), indicating that the amino acid content of fermented bean curd after thawing increased. This may be because some inactivated proteases regain activity during the thawing stage due to the rise in temperature, leading to a secondary hydrolysis of soy protein, which may result in an increase in the content of free amino acids.

## **V. Conclusion**

In this study, we investigated the changes in pH, acidity, and amino nitrogen content of low-salt fermented bean curd at different temperatures. Through the analysis of physicochemical indicators of fermented bean curd, it was found that the pH value of fermented bean curd changed at different temperatures. It exhibited a trend of initial increase followed by a decrease over time, whereas the acidity and amino nitrogen content rose gradually. The pH value ranged between 5.35 and 6.81, the total acidity varied from 0.18 g/100g to 0.38 g/100g, while the amino nitrogen content rose from approximately 0.15 g/100g to 0.31 g/100g, all of which comply with national standards. Among them, the acidity value of fermented bean curd fermented at 28°C was the highest, which was consistent with the experimental expectations. Physicochemical indicators can serve as a key indicator for evaluating fermented bean curd. For fermented bean curd after fermentation, freeze-thaw treatment was performed at -80°C, and simulated transportation was carried out at -18°C. After final air thawing at room temperature, a comparison revealed certain differences in the physicochemical indicators of the freeze-thawed fermented bean curd.

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