Synergistic Effect of the Lactic Acid Bacteria and Salt Coagulant in Improvement of Quality Characteristics and Storage Stability of Tofu

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Abstract: In this study, a new way to produce tofu with lactic acid bacteria (*Lactobacillus casei*) and salt coagulant (magnesium sulfate) has been developed and optimized in order to improve the quality characteristics and the storage stability. Processing parameters (bean-water ratio, inoculation amount, magnesium sulfate concentration and pressing time) of tofu were studied. Yield, water holding capacity (WHC), texture and sensory were measured for evaluating quality characteristics of tofu. Based on the single factor and response surface methodology (RSM), the optimized conditions of tofu were determined as follows: bean-water ratio was 1:4 g/mL, fermentation time was 5 h at 37°C when the inoculation amount was 4.0%, magnesium sulfate concentration was 2.0 mol/L and pressing time was 1 h. Under the optimum conditions, the yield of the tofu was 140.45 g, the WHC was 87.25%, the hardness was 420.36 g, and the tofu had better sensory characteristics, soft, uniform texture, as well as good flavor. The shelf life and stability of tofu during storage were also evaluated under the optimum conditions. The results showed that fermented tofu had a longer shelf life than unfermented tofu at room temperature. Compared with the “pasteurization + low temperature” group and “low temperature” group, the fermented tofu in the “microwave + low temperature” group had a longer shelf life and better-quality properties during storage. Tofu, prepared by the lactic acid bacteria fermentation and salt coagulant, would be accepted as a new type of tofu according to its quality characteristics and storage stability.

Key words: tofu, lactic acid bacteria, salt coagulant, quality characteristics, storage stability

1 Introduction

Tofu, one kind of delicious Chinese traditional soybean products, has long been a nutritional food for people in Asia, that also known as soybean curd. Tofu contains high quality protein, essential fatty acids, and no cholesterol, it could be easily favored and accepted by people. It is prepared traditionally by coagulating soy milk with a coagulant, followed by pressing to form the desirable texture. During the preparation of tofu, soy milk coagulation is usually considered be the most important step. A lot of studies using salts (CaSO₄, CaCl₂, MgCl₂ and MgSO₄), acid (glucono-δ-lactone, GDL) or enzyme (transglutaminase, TGase) as the coagulant had been reported, and previous studies suggested that coagulant can be used to improve the quality of tofu. The GDL is typically applied to make silken tofu by releasing protons slowly in the soy milk. The tofu prepared with GDL has excellent quality and WHC, but the flavor is poor. Salts dissociate rapidly at low temperature to easily make a discontinuous and weaker tofu. Tofu made by salt coagulants have tasty flavor, but short ofWHC and easily corruption. The isopeptide bond between amide groups (in a glutamine residue) and ε-amino groups (in lysine residue), could be formed by TGase, resulting in proteins assembly. Enzyme-induced tofu has the low gel strength. The species of coagulants could result the difference in physicochemical properties and flavor characteristics.

A new method to produce tofu by lactic acid bacteria (LAB) fermentation has been set up. In general, LAB is a versatile starter culture involving acidification, protein hydrolysis, flavor formation and the production of extracellular polysaccharides (EPS). These properties not only impart unique flavor, texture and nutritional health properties to the fermented product, but also enhance the preser-
The paramount factor restricting the use of LAB in the tofu production process is the weakened network. In order to overcome the shortcomings of a single coagulant, composite coagulant was used to increase the texture and sensory properties of tofu.

Our previous studies showed that tofu manufactured with L. casei and magnesium sulfate could additionally strengthen the gel network and reduce the syneresis of tofu. During the fermentation of soymilk and further gelation, the conditions such as bean-water ratio, inoculation amount, magnesium sulfate concentration and pressing time, greatly affect the aggregation behavior of protein, which is also the crucial determinant to the tofu quality.

Tofu is rich in protein, enough water and near-neutral pH value, it is easily contaminated by microorganisms even when stored at low temperature (refrigerated conditions). Therefore, tofu is a soybean product with a very short shelf-life, which indicates that extending tofu shelf-life is considered to be an important research topic.

Specific approaches had been developed by some researchers for improving tofu quality and safety, LAB fermentation is a widely practiced technology. Their bacteriostatic effects are attributed to the inhibitory metabolites formed during fermentation such as organic acid, ethanol, bacteriocins, antibacterial peptide, etc. Our previous studies showed that tofu manufactured with L. casei and magnesium sulfate could additionally strengthen the gel network and reduce the syneresis of tofu. During the fermentation of soymilk and further gelation, the conditions such as bean-water ratio, inoculation amount, magnesium sulfate concentration and pressing time, greatly affect the aggregation behavior of protein, which is also the crucial determinant to the tofu quality.

The curd was transferred to the mould (10 cm × 10 cm × 8 cm) which with filter cloth and pressed under 20 g/cm² pressure. The picture and microstructure of tofu products have been added in the Supplementary Fig. 1.

2.4 Experimental design

2.4.1 Single factor experiment design

In tofu preparation, the suitable processing parameters were selected as follow: bean-water ratio (1:3 g/mL, 1:4 g/mL, 1:5 g/mL and 1:6 g/mL), L. casei inoculation amount (2%, 4%, 6%), magnesium sulfate concentration (1.2 mol/L, 2.0 mol/L, 2.8 mol/L) and pressing time (0.5 h, 1 h, 1.5 h). The specific plan of the single factor test is in the Supplementary methods.

2.4.2 Response surface methodology

Twenty-eight experiments were performed according to RSM design with four variables and three levels for each variable. The independent variables were bean-water ratio, L. casei inoculation amount, magnesium sulfate concentration and pressing time. The response values were yield, WHC, hardness and sensory scores. The experimental design in the coded and actual levels was shown in Table 1.

2.5 Preservation test

The tofu was cut into pieces with the size of 2 cm × 2 cm × 1 cm, each of which was packaged in a package bag filled with sterile water (1:3). (1) The tofu was sterilized by pasteurization or microwave treatment. Pasteurization conditions: tofu was put in a thermostat water bath cauldron at 65°C. The central temperature of tofu measured by thermometer was 62.5~63°C and held for 30 min and cooling rapidly after sterilization. Microwave treatment conditions: the microwave power was 600 W and held for 1 min. (2) The tofu was stored at room temperature (25°C) or in a refrigerator at 4°C, respectively.

2.6 Yield

The method was adapted from the literature described by Zhu et al. The yield of tofu is the weight of tofu produced by soybean milk with the volume of 800 mL.
Tofu Prepared by the Lactic Acid Bacteria and Salt Coagulant

2.7 Water holding capacity

The method described by Cao et al.\textsuperscript{15} was adapted. The tofu samples (6 g/W) were centrifuged (7000 g, 4°C, 20 min). The supernatant was discarded and the residual liquid was carefully removed by dry filter paper. The weight of dehydrated tofu (Wt) was recorded. The water holding capacity (WHC) could be written as:

\[ WHC(\%) = \frac{Wr}{Wt} \]

2.8 Texture characteristics

The tofu was cut into a cube with the size of 1 cm × 1 cm × 1 cm through the stainless-steel knife and analyzed by the TA-XT texture analyzer (Stable Micro System, UK). In order to investigate the hardness, elasticity, cohesion adhesiveness and chewiness, the samples were compressed using P/36R probe and the total deformation of sample was 50%. The experiment conditions as follows: the trigger force was 5 g, the test speed was 5 mm/s. In addition, the speeds before and after test were 5 mm/s and 1 mm/s, respectively.\textsuperscript{16}

2.9 pH value

The pH was measured using a digital pH meter (Shanghai Yidian Scientific Instrument Co., Ltd., China).\textsuperscript{14}

2.10 Sensory characteristics

The sensory evaluation of fermented tofu samples was given by the sensory evaluation team. The shape, color, taste, flavor and acceptability of tofu were selected as the evaluation values of different tofu samples. For each sample, the results were recorded on a 5-point scale of which 1 represents the "very unacceptable", 2 represents "unacceptable", 3 represents "acceptable", 4 represents "medium-liked" and 5 represents "very liked". Evaluation steps, scoring standards and details are as shown in the Supplementary methods 2.

2.11 Determination of the colonies total number of fermented tofu

In order to determine the colonies total number, tofu (10 g) was homogenized with 90 mL of 0.85% sterile saline (8.5 g/L of sodium chloride) for 2 min, the homogenate (1 mL) was serially diluted tenfold with sterile saline (9 mL). Then, diluted solution (1 mL) was spread into a sterile plate and plate count agar medium (about 20 mL) was poured into the plate immediately. The plate was cultured at 37°C for 48 h, and colonies total number were determined as colony forming units (CFU) per g.

2.12 Statistical analysis

The data were presented as mean ± standard deviation and all tests were repeated at least triplicate. SPSS 20.0 software was used to analyze the statistical results. The analysis of variance and significance (p<0.05) was performed through the Duncan’s test. The Origin 8.5 was used for drawing processing and the response surface analysis was performed with the design-expert v 8.0.6 software.

3 Results and Discussion

3.1 The pH of tofu

As shown in Fig. 1a, the pH value decreased significantly due to the increasing and accumulation in acid yield production with extend the fermentation time. When the fermentation time was increased from 2 h to 3 h, the pH dropped sharply, it may be inferred that the strain in the logarithmic growth phase had strong acid-producing ability. The pH decreased slowly in the later stage of fermentation period, which can be attribute to the acid inhibits the growth of L. casei. It can be seen from Fig. 1b that there were no significant differences among the pH value in the four different ratios of bean to water. The pH was continuously decreased, so that the soybean protein could be solidified. The pH value decreased with increasing of the inoculation amount (Fig. 1c) owing to more lactic acid was produced. The metabolic activity and growth requirements of the strain are dependent on pH changes. The low pH after fermentation is considered to be a protective factor that inhibits spoilage bacteria.\textsuperscript{16}

3.2 Yield and WHC of the tofu

The yield of tofu related to water, uncoagulated proteins,
and other soluble solids to be expelled from the gel during the pressing process of tofu production. The yield showed a significant decrease with the increase of bean-water ratio as shown in Fig. 2a. The yield of tofu (162.47 g) was significantly higher than other groups when the bean-water ratio was 1:3 g/mL. Presumably, this ratio (1:3 g/mL) made the highest soluble solids and protein concentration of the soybean milk. WHC had no significant difference when bean-water ratio was 1:3, 1:4 or 1:5 g/mL, while all were lower than the group when the bean-water ratio was 1:6 g/mL. The effect of inoculation amount on the yield and WHC of tofu was shown in Fig. 2b. There was no significant difference in yield and WHC when the inoculation amount from 2 to 4. The magnesium sulfate concentrations had significant effect on WHC of tofu (Fig. 2c). WHC increased firstly and then decreased, when the concentration was 2 mol/L, the WHC was highest, which was 88.59%. At lower coagulant salt concentration (1.2 mol/L), the interaction of soybean protein and magnesium ions was not sufficient to form strong tofu, so water was easily released from the gel structure. When the magnesium sulfate concentration (2 mol/L) was appropriate, a better network gel structure was formed. When the magnesium sulfate concentration increased to 2.8 mol/L, protein aggregation was accelerated, large aggregates and excessive cross-linking were formed, the uneven and rough gel structure resulted in reduced WHC. The yield decreased significantly with the increase of pressing time (Fig. 2d). When the pressing time was 0.5 h, the yield reached the maximum value (144.59 g). It was indicated that the water outflow in the tofu increased due to pressing, resulting in the decrease of yield. When the pressing time was 1.5 h, the WHC of tofu was significantly higher than the other two groups (p < 0.05), which may be the tighter structure of the protein under the long pressing time resulted in water content reduction, so that WHC increased.

3.3 Texture characteristics of the tofu

The hardness of tofu decreased firstly and then increased with the bean-water ratio increased (Table 2a). The decrease in hardness maybe due to the concentration of protein decreased. The high protein concentration increased the likelihood and frequency of protein particle aggregation, and formed high strength gel-network structure. Due to the more cross-linking reactions between coagulant and protein when the bean-water ratio was 1:6 g/mL, the denser protein matrix formed. Syneresis resulted the reduction of yield and the increase of WHC and hardness, the result was consistent with the acquired highest WHC (bean-water ratio was 1:6 g/mL) (Fig. 2c). It can be seen from Table 2b that the hardness increased as inoculation amount increased. The trends of cohesion and chewiness were the same as hardness. The denser gel network formed due to more lactic acid produced when the inoculation amount was high, and increased the cross-linking of protein. The effect of magnesium sulfate concentrations on the texture properties of tofu were shown in Table 2c. When the magnesium sulfate concentration was 2.0 mol/L, the hardness (407.84 g) of the tofu was significantly higher than the value of 1.2 mol/L. The magnesium sulfate con-
Tofu Prepared by the Lactic Acid Bacteria and Salt Coagulant

J. Oleo Sci.

Concentration (2.0 mol/L) accelerates the coagulation rate of soybean protein and leads to the dense and compact structure, which increased the hardness\(^{23}\), but the further increase of magnesium sulfate concentration resulted in the fast coagulation rate and rough tofu structure, which was the reason of low hardness. Cohesion was the lowest at magnesium sulfate concentration of 2.8 mol/L. Chewiness was the highest at a magnesium sulfate concentration of 2.0 mol/L. The hardness increased significantly as the pressing time increased (Table 2d). When the pressing time was 1.5 h, the tofu had the highest hardness and its yield was 462.34 g. The long pressing time reduced water,
removed some carbohydrates and increased the protein concentration, and then lead to a dense protein network, resulting in increased hardness\(^a\).

### 3.4 Sensory characteristics

It can be seen from Table 3a that the bean-water ratio had no significant influence on the appearance and color of the fermented tofu. When the bean-water ratio was 1:4 g/mL, the taste and flavor of tofu were the highest, so the tofu was most popular among consumers. The taste, acceptability and the total score were the highest when the inoculation amount was 4% (Table 3d). Wang et al. found that 4% of the inoculation amount has a better taste for soybean curd, which is consistent with the results of this test\(^7\). Lactic acid, produced by 4% lactic acid bacteria, provided the sufficient time to protein rearrangement, and the rearrangement affected the sensory of tofu. The sensory characteristics of tofu were the best of all when the magnesium sulfate concentration of 2.0 mol/L or the pressing time of 1.0 h (Tables 3c and 3d). The tofu would be poor in softness due to the lack of moisture when the pressing time was too long, so the tofu was not liked by consumers.

### 3.5 Analysis of RSM and optimizations

The response surface methodology design and experimental results were shown in Tables 4 and 5. More detailed data were shown in the Appendix. The regression equations of yield\((Y_1)\), WHC\((Y_2)\), hardness\((Y_3)\), sensory\((Y_4)\) versus independent variable A (bean-water ratio), B (inoculation amount), C (magnesium sulfate concentration), D (pressing time) were obtained.

The regression equation for the yield \(Y_1\) was:

\[
Y_1 = 129.48 + 17.72A + 0.60B + 0.004167C - 4.89D + 2.81
\]

The regression equation for WHC \(Y_2\) was:

\[
Y_2 = 90.98 - 1.19A + 1.04B - 1.31C + 1.15D - 2.82AB - 0.84AC + 0.99AD - 1.26BC - 1.64BD + 2.8CD - 2.01
\]

The regression equation for hardness \(Y_3\) was:

\[
Y_3 = 425.08 + 10.81A + 20.20B + 12.51C + 14.01D - 2.93
\]

The regression equation for sensory \(Y_4\) was:

\[
Y_4 = 26.22 + 0.49A + 0.69B + 0.43C - 0.46D - 0.55AB + 0.65AC + 0.56AD - 0.49BC - 0.85BD + 0.075CD -
\]
The results showed that the regression models $Y_1$, $Y_2$, $Y_3$, and $Y_4$ were extremely significant ($p<0.01$), and the lack of fit were not significant, respectively 0.0734, 0.8621, 0.8725, 0.1495, indicating that the regression equation had a high degree of fit and small error. The coefficient of determination ($R^2$) of the $Y_1$ model was 0.9766, which means that only 2.34% of the total variation was not explained by this model; the adjusted $R^2$ (0.9598) was quite close to the $R^2$, indicating that the developed model fitted well. The high values of $R^2$, adjusted $R^2$ confirmed the accuracy of the model in showing the relationship between yield ($Y_1$), WHC ($Y_2$), hardness ($Y_3$), sensory ($Y_4$), and variables. This model could be used to analyze and predict the effects of processing factors on the quality characteristics of fermented tofu. Through one-term analysis, the effect of bean-water ratio and pressing time on the yield of tofu were extremely significant, but inoculation amount and magnesium sulfate concentration were not significant. At the same time, the degree of bean-water ratio, inoculation amount, magnesium sulfate concentration, pressing time, and sensory characteristics were significant.

Through the regression equation, the best fermentation tofu process conditions were obtained through the soft-

| NO. | A   | B   | C   | D   | $Y_1$ | $Y_2$ | $Y_3$ | $Y_4$ |
|-----|-----|-----|-----|-----|-------|-------|-------|-------|
| 1   | -1  | 0   | -1  | 0   | 109.44| 88.0122| 353.804| 21.24 |
| 2   | 1   | 0   | 0   | 1   | 138.27| 90.7962| 419.352| 22.67 |
| 3   | 0   | 1   | 0   | -1  | 137.15| 92.8445| 436.317| 23.76 |
| 4   | 0   | 1   | -1  | 0   | 129.64| 89.9318| 399.282| 23.98 |
| 5   | 1   | 0   | 0   | -1  | 153.47| 86.4801| 466.87 | 21.78 |
| 6   | 0   | -1  | -1  | 0   | 128.625| 85.5871| 366.484| 21.01 |
| 7   | 0   | 0   | 0   | 0   | 131.281| 88.7561| 418.289| 24.00 |
| 8   | 0   | -1  | 0   | -1  | 137.27| 86.3788| 387.32 | 21.01 |
| 9   | 0   | 0   | 0   | 0   | 128.282| 89.779 | 440.43 | 23.22 |
| 10  | 0   | 0   | 0   | 0   | 128.283| 92.735 | 430.288| 23.23 |
| 11  | 1   | 0   | 1   | 0   | 143.35| 81.4508| 409.496| 23.03 |
| 12  | 1   | -1  | 0   | 0   | 141.69| 88.2052| 418.289| 21.67 |
| 13  | 0   | 1   | 0   | 1   | 130.35| 89.2784| 440.065| 20.72 |
| 14  | -1  | -1  | 0   | 0   | 109.38| 86.4551| 420.235| 20.32 |
| 15  | 0   | 0   | 0   | 1   | 126.04| 90.7043| 420.771| 22.53 |
| 16  | 0   | 0   | 0   | 1   | 137.57| 80.1535| 367.512| 23.55 |
| 17  | -1  | 1   | 0   | 0   | 108.79| 93.3264| 466.372| 22.31 |
| 18  | 0   | 0   | 0   | 0   | 130.284| 90.984 | 418.95 | 23.81 |
| 19  | 1   | 0   | -1  | 0   | 145.07| 86.2052| 434.03 | 21.23 |
| 20  | 0   | -1  | 1   | 0   | 130.815| 86.2482| 380.987| 22.51 |
| 21  | -1  | 0   | 0   | -1  | 118.07| 88.2085| 366.209| 21.52 |
| 22  | -1  | 0   | 1   | 0   | 108.73| 86.6338| 433.343| 20.42 |
| 23  | 0   | 0   | -1  | -1  | 137.75| 88.8356| 363.173| 22.13 |
| 24  | 0   | 1   | 1   | 0   | 130.82| 85.5605| 459.964| 23.54 |
| 25  | 0   | 0   | 0   | 0   | 129.28| 92.643| 416.775| 23.83 |
| 26  | -1  | 0   | 0   | 1   | 107.16| 88.575 | 431.103| 20.18 |
| 27  | 0   | 0   | -1  | 1   | 126.75| 87.9003| 405.238| 20.81 |
| 28  | 1   | 1   | 0   | 0   | 152.33| 83.7787| 452.711| 21.67 |

A-Bean-water ratio (g/mL), B-Inoculation amount (%), C-Magnesium sulfate concentration (mol/L), D-Pressing time (h), $Y_1$-Yield (g), $Y_2$-WHC (%), $Y_3$-Hardness (g), $Y_4$-Sensory scores.
ware Design-Expert 8.0.6 with the largest yield, hardness, WHC and the highest sensory score. The optimal preparation conditions were: the bean-water ratio was 1:3.57 g/mL, the inoculation amount was 4.5\%, the magnesium sulfate concentration was 1.83 mol/L, and the pressing time was 0.77 h. The predicted yield was 137.46 g, the WHC was 90.76\%, the hardness was 418.96 g and the sensory score was 24.94. According to the actual operability, the optimal process was determined as follows: the bean-water ratio was 1:4 g/mL, the inoculation amount was 4\%, the magnesium sulfate concentration was 2 mol/L, and the pressing time was 1 h. Under the optimal conditions, the measured yield of fermented tofu was 140.45 ± 3.5 g, WHC was 87.25 ± 1.25\%, hardness was 420.36 ± 35.28 g, sensory score was 24 ± 0.65, the actual value was closed to the predicted value and the deviation was small, which proved the equation was accurate and reliable.

### 3.6 Storage stability

The colonies total number of the fermented tofu and unfermented tofu (placed at room temperature) were compared in Table 6.

The colonies total number of fermented tofu were significantly lower than unfermented tofu. After a day, the colonies total number of unfermented tofu were reached 10^5, but L. casei fermented tofu reached 10^5 after 3 days, the results showed that shelf life could be extended by L. casei fermentation. Serrazanetti et al. found that the fermentation of L. casei and Lactobacillus acidophilus could prevent or delay the growth of spoilage microorganisms, increase product stability and improve quality of tofu\(^\text{5}\). It may be due to some antibacterial substances were pro-

### Table 5 Variance analysis of response surface methodology.

| Source | Y\(_1\) | Y\(_2\) | Y\(_3\) | Y\(_4\) |
|--------|--------|--------|--------|--------|
| Model  | \(<0.0001\) ** | 0.0008 ** | 0.0005 ** | \(<0.0001\) ** |
| A      | \(<0.0001\) ** | 0.0265 * | 0.0346 * | 0.0108 * |
| B      | 0.4164 | 0.0485 * | 0.0006 ** | 0.0009 ** |
| C      | 0.9955 | 0.0163 * | 0.017 * | 0.0208 * |
| D      | \(<0.0001\) ** | 0.0318 * | 0.0089 ** | 0.0153 * |
| AB     | 0.0413 * | 0.0044 ** | 0.7197 | 0.0748 |
| AC     | 0.8428 | 0.3275 | 0.0058 ** | 0.0386 * |
| AD     | 0.4052 | 0.255 | 0.0034 ** | 0.0725 |
| BC     | 0.8428 | 0.1527 | 0.1708 | 0.1132 |
| BD     | 0.4856 | 0.068 | 0.1562 | 0.0107 * |
| CD     | 0.9171 | 0.0039 ** | 0.7316 | 0.7976 |
| A\(^2\) | 0.0109 * | 0.0081 ** | 0.261 ** | \(<0.0001\) ** |
| B\(^2\) | 0.1448 | 0.1874 | 0.3001 | \(<0.0001\) ** |
| C\(^2\) | 0.6021 | 0.0001 ** | 0.0007 ** | \(<0.0001\) ** |
| D\(^2\) | 0.0064 ** | 0.3852 | 0.185 | \(<0.0001\) ** |
| Lack of fit | 0.0734 | 0.6131 | 0.1419 | 0.1495 |

\(R^2=0.9766\) | \(R^2_{\text{Adj}}=0.9598\) | \(R^2=0.8621\) | \(R^2_{\text{Adj}}=0.7242\) | \(R^2=0.8725\) | \(R^2_{\text{Adj}}=0.7450\) | \(R^2=0.9573\) | \(R^2_{\text{Adj}}=0.9146\)

A-Bean-water ratio (g/mL), B-Inoculation amount (%), C-Magnesium sulfate concentration (mol/L), D-Pressing time (h), Y\(_1\)-Yield (g), Y\(_2\)-WHC (%), Y\(_3\)-Hardness (g), Y\(_4\)-Sensory scores.

### Table 6 Effect of storage time on the total number of colonies of two tofu.

| Time (d) | Total number of colonies (CFU/g) |
|----------|---------------------------------|
|          | 1                              | 2                              | 3                              |
| Unfermented tofu | 1.2 ± 0.18 × 10^5 | – | – |
| Fermented tofu | 2.3 ± 0.43 × 10^5 | 1.5 ± 0.22 × 10^4 | 3.2 ± 0.80 × 10^5 |

\(^{a-b}\) Means in a same column and index with different letters are significantly different (\(p < 0.05\)).
duced during the fermentation of lactic acid bacteria such as organic acids, bacteriocins or other anti-corruption compounds.

The colonies total number of fermented tofu under different treatment conditions were shown in Fig. 3a. "PL", "ML" group could significantly prolong the shelf life of fermented tofu. The bactericidal effect of the "ML" group was better than the "PL" group. Pasteurization is a heat treatment that causes changes in microbial cell membrane structure, protein denaturation, enzyme inactivation and direct and indirect DNA damage, which could extend the shelf life and preserve the nutrient and flavor characteristics in foods. Microwave is a combination of convection and radiant heating. It is the result of the combination of thermal and non-thermal effects. The thermal effect mainly acts as a rapid temperature-increasing bactericidal. The non-thermal effect is to change the protein and physiologically active substances in the microorganisms so that the microbial cells lose their vitality or die. So, the bactericidal effect of the microwave was better than pasteurization. It can be seen from Figs. 3b and 3c that the WHC and hardness of the three treatment groups showed a downward trend with the prolongation of storage time. The WHC of the "ML" group was significantly higher, the result indicated that the short microwave sterilization time caused the small damage to the protein structure, as well as microwave sterilization didn’t destroy the biologically active ingredients. Within 3~9 days, the hardness of low temperature treatment group decreased slowly (Fig. 3c), which may be the pasteurization treatment had a certain destructive effect on the internal microstructure of the food. As shown in Fig. 3d, the sensory characteristics showed a downward trend as the storage time extended. The sensory characteristics of tofu in the "L" group decreased significantly. The increased bacteria reduced the sensory characteristics. Compared with long-term pasteurization treatment, short-time microwave treatment has high sterilization efficiency, and can better preserve the original flavor of fermented tofu.

4 Conclusions

The improvement of tofu quality characteristics and storage stability is a key issue in the production of tofu. In this study, optimal process conditions of fermented tofu (L. casei with magnesium sulfate) were obtained through the single factor and RSM model. In addition, the shelf life was extended. Bean-water ratio, inoculation amount, magnesium sulfate concentration and pressing time could significantly improve the quality characteristics of tofu. Compared with the unfermented tofu, tofu prepared by fermentation contained more antibacterial substances, which reduced the reproduction ability of harmful bacteria in tofu. The storage property of the "PL" group and the "ML" group was superior to the "L" group. The fermented tofu with the best quality and sensory characteristics during the preservation period was the "ML" group. Furthermore, it was found that Lactobacillus fermentation could improve the storage stability of tofu, good estimates of the storage stability of tofu prepared by LAB fermentation and salt coagulant offer the potential for wider use of this product.

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Conflict of interest

The authors have declared no conflict of interest.

Supporting Information

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