Optimization of Microbial Solid-State Fermentation Process for Soybean Meal and Changes in Nutrient Content (Postprint)

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Abstract

This study optimized the process conditions for solid-state fermentation of soybean meal by Bacillus amyloliquefaciens alone and by a mixed culture of three strains (Bacillus amyloliquefaciens, Lactobacillus plantarum, and Saccharomyces cerevisiae), using small peptide content as an indicator, and investigated changes in nutrient content before and after fermentation. The optimal inoculation time for the three test strains into the solid-state medium was determined through their growth curves. A single-factor experimental design was employed to investigate the effects of four factors (Bacillus amyloliquefaciens inoculum size, temperature, material-to-water ratio, and fermentation time) on small peptide production from soybean meal fermentation, and based on this, a four-factor three-level orthogonal experimental design was used to optimize the process conditions for both single-strain and mixed-culture solid-state fermentation of soybean meal. The nutrient content of soybean meal, glycinin content, protein molecular weight, and pH of the fermentation product were measured before and after fermentation. The results showed that the optimal time for inoculating the three test strains into the solid-state medium was after 21 h of expansion culture in their respective seed media. The optimal process conditions for single-strain solid-state fermentation of soybean meal by Bacillus amyloliquefaciens were: inoculum size of 10%, temperature of 40 °C, material-to-water ratio of 1.0:1.2, and fermentation time of 72 h; the optimal process conditions for mixed-culture solid-state fermentation of soybean meal by Bacillus amyloliquefaciens, Lactobacillus plantarum, and Saccharomyces cerevisiae were: inoculum size of 15%, temperature of 31 °C, material-to-water ratio of 1.0:1.0, and fermentation time of 120 h, with an inoculation ratio of the three strains of Bacillus amyloliquefaciens:Lactobacillus plantarum:Saccharomyces cerevisiae = 9:3:2. After microbial fermentation, the contents of small peptides, crude protein, crude ash, and crude fat in the fermentation product were significantly

increased compared with those before fermentation (P<0.05), while the crude fiber content was significantly decreased (P<0.05). The glycinin content in the fermentation products of both the single-strain and mixed-culture fermentation groups was significantly lower than that in the unfermented group (P<0.05). The protein molecular weight in the fermentation products of both the single-strain and mixed-culture fermentation groups was reduced compared with the unfermented group. The pH of the fermentation product in the mixed-culture fermentation group was significantly lower than that in the unfermented group (P<0.05), whereas the pH in the single-strain fermentation group did not differ significantly from the unfermented group (P>0.05). In summary, the nutritional value of soybean meal was improved to a certain extent after microbial solid-state fermentation, large-molecular-weight proteins were degraded, and pH changes occurred, with differences observed between single-strain and mixed-culture fermentation effects.

Full Text

Process Optimization of Solid-State Fermentation of Soybean Meal by Microorganisms and Its Nutrient Changes

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Abstract: This experiment optimized the process conditions for solid-state fermentation of soybean meal by *Bacillus amyloliquefaciens* alone and by a mixed culture of *Bacillus amyloliquefaciens*, *Lactobacillus plantarum*, and *Saccharomyces cerevisiae*, using small peptide content as the primary indicator, and investigated changes in nutrient composition before and after fermentation. The optimal inoculation time for the solid-state fermentation medium was determined based on the growth curves of the three test strains. Single-factor experiments examined the effects of four variables—inoculation amount, temperature, feed:water ratio, and fermentation time—on small peptide production from soybean meal fermented by *B. amyloliquefaciens*. Building on these results, orthogonal experimental designs were employed to optimize the fermentation process conditions for both single-strain and mixed-strain fermentation. Nutrient contents, soybean globulin content, protein molecular weight, and pH of the fermentation products were measured before and after fermentation. The results showed that the optimal inoculation time for all three strains was 21 h after expansion in their respective seed media. The optimal conditions for single-strain fermentation by *B. amyloliquefaciens* were: 10% inoculation amount, 40°C temperature, 1.0:1.2 feed:water ratio, and 72 h fermentation time. The optimal conditions for mixed-strain fermentation were: 15% inoculation amount, 31°C temperature, 1.0:1.0 feed:water ratio, and 120 h fermentation time, with a strain

inoculation ratio of *B. amyloliquefaciens*:*L. plantarum*:*S. cerevisiae* = 9:3:2. After microbial fermentation, the contents of small peptides, crude protein, ash, and ether extract increased significantly ($P<0.05$), while crude fiber content decreased significantly ($P<0.05$). Soybean globulin content in both single-strain and mixed-strain fermentation groups was significantly lower than in the unfermented group ($P<0.05$). Protein molecular weight in the fermented products was reduced compared to the unfermented soybean meal. The pH of the mixed-strain fermentation product was significantly lower than that of the unfermented group ($P<0.05$), while the pH of the single-strain fermentation product showed no significant difference from the unfermented group ($P>0.05$). In conclusion, solid-state fermentation by microorganisms improved the nutritional value of soybean meal to a certain extent, degraded macromolecular proteins, and altered pH, with distinct differences observed between single-strain and mixed-strain fermentation effects.

Keywords: soybean meal; single strain; mixed strains; fermentation; process optimization; nutrients

Introduction

Soybean meal serves as a crucial plant-based protein source in China. Compared with other plant protein sources such as cottonseed meal, rapeseed meal, and peanut meal, soybean meal offers a more balanced amino acid composition, higher digestibility, and better palatability [1]. Relative to animal protein feeds like fish meal, it provides advantages including more abundant resources, lower cost, reduced susceptibility to oxidative spoilage, and higher safety margins [2]. However, as a major livestock production country, China still faces prominent supply-demand contradictions regarding soybean meal resources. Additionally, soybean meal contains anti-nutritional factors including soybean globulin, trypsin inhibitors, and phytic acid [3]. Soybean globulin accounts for 40% of total protein [4] and represents the most abundant globulin in soybeans as well as one of the most heat-stable antigenic proteins. It constitutes a primary component causing allergic reactions and diarrhea in animals, limiting soybean meal usage in diets and posing serious hazards to livestock and poultry. Therefore, adopting appropriate technological approaches to enhance the feed value of existing soybean meal resources holds significant importance.

In recent years, increasing attention has focused on microbial fermentation technology, particularly using beneficial bacteria, for feed production. *Bacillus amyloliquefaciens*, a probiotic bacterium, exhibits rapid reproduction, good stability, strong viability [5], and produces abundant amylase, protease, and cellulase [6-9], demonstrating promising results in solid-state fermentation of soybean meal [10-12]. Although numerous studies have reported on fermentation process parameters for soybean meal, strict evaluation standards remain necessary for strain resources and fermentation processes, and comparative studies between

single-strain and mixed-strain fermentation are limited. Therefore, this study aimed to optimize process parameters for both single-strain fermentation by *B. amyloliquefaciens* and mixed-strain fermentation in combination with *L. plantarum* and *S. cerevisiae*, while analyzing and comparing the physicochemical properties of soybean meal before and after fermentation to provide scientific evidence for strain selection and process optimization in fermented soybean meal production.

Materials and Methods

1.1 Experimental Materials and Strains

Soybean meal and wheat bran were provided by the Changping Nankou Base of the Feed Research Institute, Chinese Academy of Agricultural Sciences, and were ground to pass through a 40-mesh sieve. Sterile water was prepared by dispensing distilled water and sterilizing at 121°C for 20 min.

Bacillus amyloliquefaciens was isolated and screened in our laboratory. *Lactobacillus plantarum* (preservation number 1.557) and *Saccharomyces cerevisiae* (preservation number 2.388) were purchased from the China General Microbiological Culture Collection Center.

1.2 Culture Media

1.2.1 Liquid Seed Media LB medium contained 10.0 g NaCl, 10.0 g peptone, and 5.0 g yeast extract in 1,000 mL distilled water, adjusted to pH 7.4 and sterilized at 121°C for 20 min. MRS medium contained 20.0 g glucose, 10.0 g peptone, 8.0 g beef extract, 4.0 g yeast extract, 0.5 g MgSO , 0.3 g MnSO , 2.0 g ammonium citrate, 5.0 g sodium acetate, and 1.0 mL Tween-80 in 1,000 mL distilled water, adjusted to pH 6.2-6.6 and sterilized at 121°C for 20 min. YPD medium contained 20.0 g glucose, 10.0 g peptone, and 5.0 g yeast extract in 1,000 mL distilled water at natural pH, sterilized at 121°C for 20 min.

1.2.2 Slant Media Each liquid seed medium was supplemented with 20.0 g agarose to prepare slant media.

1.2.3 Solid Fermentation Medium The solid fermentation medium consisted of 45.0 g soybean meal and 5.0 g wheat bran with appropriate sterile water at natural pH.

1.3 Experimental Procedures

1.3.1 Preparation of Fermentation Seed Liquid A loopful of each strain was inoculated from slant media into respective liquid seed media: LB for *B. amyloliquefaciens*, MRS for *L. plantarum*, and YPD for *S. cerevisiae*. *B. amyloliquefaciens* was cultured at 37°C with shaking at 180 rpm, *S. cerevisiae* at

30°C with shaking at 180 rpm, and *L. plantarum* at 30°C under static conditions for 48 h. These cultures were then transferred at 1% inoculation amount to fresh liquid seed media for 24 h expansion to prepare fermentation seed liquids.

1.3.2 Growth Curve Determination and Inoculation Time Selection Growth curves were established using spectrophotometric turbidity method [13]. Sterile uninoculated liquid seed media served as blank controls. Absorbance at 600 nm was measured at 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, and 48 h under respective culture conditions. Growth curves were plotted with culture time as the x-axis and absorbance as the y-axis. Strains in the logarithmic growth phase, when cell vitality is strongest and growth most vigorous [14], were selected for inoculation into solid fermentation media.

1.3.3 Solid-State Fermentation Method Solid fermentation medium was dispensed into 250 mL Erlenmeyer flasks. Prepared fermentation seed liquid was inoculated at specified amounts into the soybean meal-containing solid medium, mixed thoroughly, and fermented statically.

1.4 Experimental Design

1.4.1 Single-Strain Fermentation of Soybean Meal Single-Factor Experiments: Four factors—inoculation amount (A), temperature (B), feed:water ratio (C), and fermentation time (D)—were investigated in single-factor experiments using small peptide content as the indicator to evaluate individual factor effects on peptide production by *B. amyloliquefaciens*.

Process Optimization: Based on single-factor results, an L (3) orthogonal experimental design was employed to optimize the fermentation process for single-strain fermentation, with small peptide content as the indicator. Each level had three replicates. The orthogonal experimental design is shown in Table 1 .

1.4.2 Mixed-Strain Fermentation of Soybean Meal Strain Ratio Optimization: An L (3) orthogonal design was used to optimize the inoculation ratio of the three strains across three levels, with three replicates per level. Fermentation was conducted at 34°C, 1.0:1.0 feed:water ratio, and natural pH for 48 h, using small peptide content as the indicator. The experimental design is presented in Table 2 .

Process Condition Optimization: Based on the optimal strain ratio, an L (4) orthogonal design was used to optimize four factors—inoculation amount (A), temperature (B), feed:water ratio (C), and time (D)—each at four levels with three replicates. The experimental design is shown in Table 3 .

1.5 Measurement Indicators and Methods

After fermentation, products were dried to constant weight at 50°C, conditioned indoors for 24 h, ground to pass through a 60-mesh sieve, and analyzed.

1.5.1 Small Peptide and Routine Nutrient Content Determination Small peptide content was measured according to the light industry standard "Soybean Peptide Powder" (QB/T 2653-2004). Crude protein content was determined following the national standard "Determination of Crude Protein in Feed" (GB/T 6432-1994). Crude fiber content was measured per "Determination of Crude Fiber Content in Feed—Filtration Method" (GB/T 6434-2006). Ash content was analyzed according to "Determination of Crude Ash in Feed" (GB/T 6438-2007). Ether extract content was determined following "Determination of Crude Fat in Feed" (GB/T 6433-2006).

1.5.2 Protein Molecular Weight Determination Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [15] was used to determine protein molecular weight. One gram of 60-mesh soybean meal was extracted with 0.1 mol/L Tris-HCl buffer (pH 8.0) for 1 h, centrifuged at 3,000×g for 10 min at 4°C, and the supernatant stored at 4°C. Electrophoresis employed 5% stacking gel and 15% separating gel, with 20 L supernatant loaded per lane, run at 20 mA and 80 V for 2 h, followed by Coomassie brilliant blue staining.

1.5.3 Soybean Globulin Content Determination A soybean globulin detection kit was used based on indirect competition principle. Sample soybean globulin competes with pre-coated antigens for antibodies, followed by enzyme-labeled secondary antibody addition and TMB substrate development. Sample absorbance correlates negatively with soybean globulin content, allowing quantification via microplate reader and standard curve comparison.

1.5.4 pH Determination of Fermented Soybean Meal Three grams of dried fermented soybean meal were mixed with 30.0 mL distilled water, stirred evenly, held at 4°C for 6 h, filtered, and the pH of the filtrate measured with a pH meter.

1.6 Statistical Analysis

Experimental data were preliminarily processed using Excel 2016 and statistically analyzed with SPSS 19.0 software. Orthogonal experimental data underwent range and variance analysis using general linear model univariate procedures, while other data were analyzed by one-way ANOVA to test for significant differences among groups, followed by Duncan' s multiple comparison. Results are expressed as "mean ± standard deviation" with significance level set at $P<0.05$.

Results

2.1 Growth Curves and Inoculation Time Determination

2.1.1 Growth Curve of *Bacillus amyloliquefaciens* As shown in Figure 1 [Figure 1: see original paper], *B. amyloliquefaciens* reached its most vigorous growth phase at 33 h of cultivation at 37°C, with rapid growth occurring between 18-24 h, identified as the logarithmic growth phase.

2.1.2 Growth Curve of *Lactobacillus plantarum* Figure 2 [Figure 2: see original paper] shows that *L. plantarum* grew rapidly during 3-21 h at 30°C, peaked at 27 h, then grew slowly with decreasing growth rate after 30 h.

2.1.3 Growth Curve of *Saccharomyces cerevisiae* Figure 3 [Figure 3: see original paper] indicates that *S. cerevisiae* grew rapidly during the first 24 h at 30°C, reached peak growth at 27 h, and subsequently entered a relatively stable phase.

2.1.4 Determination of Inoculation Time To compare the growth cycles of the test strains, growth curves were combined in Figure 4 [Figure 4: see original paper]. *Lactobacillus plantarum* grew most rapidly, entering the stationary phase after 21 h, while *B. amyloliquefaciens* and *S. cerevisiae* had longer cycles, entering stationary phase at 27 h and 24 h, respectively. To standardize procedures and simplify mixed fermentation operations, 21 h was selected as the inoculation time for all three strains, as all were in the logarithmic growth phase with strong vitality and geometric growth rates, ensuring rapid establishment in the solid medium.

2.2 Single-Factor Experiment Results

2.2.1 Effect of Inoculation Amount on Small Peptide Content Figure 5 [Figure 5: see original paper] shows that under conditions of 1.0:1.0 feed:water ratio, 35°C temperature, and 72 h fermentation time, different inoculation amounts produced varying effects. With 0% inoculation, small peptide content was 1.11%, significantly lower than other amounts ($P<0.05$). As inoculation amount increased, small peptide content first increased then decreased, reaching maximum (11.14%) at 10% inoculation. Contents at 15% and 20% inoculation were identical.

2.2.2 Effect of Feed:Water Ratio on Small Peptide Content Figure 6 [Figure 6: see original paper] demonstrates that at 10% inoculation, 35°C, and 72 h fermentation, different feed:water ratios affected fermentation differently. At 1.0:0.4 ratio, small peptide content was 5.71%, significantly lower than other ratios ($P<0.05$). With increasing water content, small peptide content increased gradually, peaking at 11.57% at 1.0:0.8 ratio, then declining with further water increase.

2.2.3 Effect of Temperature on Small Peptide Content Figure 7 [Figure 7: see original paper] reveals that at 10% inoculation, 1.0:1.0 feed:water ratio, and 72 h fermentation, different temperatures influenced fermentation outcomes. At 45°C, small peptide content was lowest (6.54%), significantly lower than other temperatures ($P<0.05$). Maximum content (11.06%) occurred at 30°C, though no significant differences were observed among 25°C, 30°C, 35°C, and 40°C ($P>0.05$).

2.2.4 Effect of Fermentation Time on Small Peptide Content Figure 8 [Figure 8: see original paper] shows that at 10% inoculation, 1.0:1.0 feed:water ratio, and 35°C, fermentation time significantly affected results. At 24 h, small peptide content was 6.30%, significantly lower than other durations ($P<0.05$). Maximum content (10.37%) occurred at 72 h, with no significant differences from 48 h, 96 h, or 120 h ($P>0.05$).

2.3 Optimization of Single-Strain Fermentation Conditions

Range analysis (R values) of orthogonal results in Table 4 showed the influence order of four factors on small peptide production: B>D>C>A, with temperature having the greatest effect, followed by fermentation time, while feed:water ratio and inoculation amount had smaller effects. Inoculation amount showed minimal influence and was treated as error term in variance analysis. Variance analysis (Table 5) revealed significant effects of temperature and fermentation time on small peptide content ($P<0.05$). Combined with k-value analysis, the optimal condition combination was A2B3C3D2: 10% inoculation, 40°C temperature, 1.0:1.2 feed:water ratio, and 72 h fermentation time.

2.4 Optimization of Mixed-Strain Fermentation Conditions

2.4.1 Strain Ratio Optimization Results Range analysis (R values) in Table 6 indicated the influence order of three strains: A>B>C, with *B. amyloliquefaciens* having the greatest impact, followed by *L. plantarum*, and *S. cerevisiae* having the smallest effect. Variance analysis (Table 7) showed no significant effects of individual strains on small peptide content ($P>0.05$). The optimal strain ratio combination was A3B3C2, corresponding to *B. amyloliquefaciens*:*L. plantarum*:*S. cerevisiae* = 9:3:2.

2.4.2 Process Condition Optimization Results Range analysis (R values) in Table 8 revealed the influence order: A>D>C>B, with inoculation amount having the greatest effect, followed by fermentation time, while feed:water ratio and temperature had minimal effects. Variance analysis (Table 9) showed significant effects of inoculation amount and fermentation time ($P<0.05$). Combined with k-value analysis, the optimal condition combination was A4B1C3D4: 15% inoculation, 31°C temperature, 1.0:1.0 feed:water ratio, and 120 h fermentation time.

2.5 Changes in Soybean Meal Before and After Fermentation

2.5.1 Nutrient Content Changes Under optimal conditions, nutrient changes are shown in Table 10 . Small peptide contents after single-strain (*B. amyloliquefaciens*) and mixed-strain (9:3:2 ratio) fermentation were 12.73% and 10.42%, respectively, both significantly higher than unfermented meal ($P<0.05$), with single-strain fermentation producing significantly more peptides than mixed-strain ($P<0.05$). Crude protein increased from 46.70% in unfermented meal to 55.31% (single-strain) and 56.14% (mixed-strain) ($P<0.05$), with mixed-strain significantly higher than single-strain ($P<0.05$). Crude fiber decreased significantly after both fermentations ($P<0.05$) with no difference between groups ($P>0.05$). Ash content increased from 7.58% to 9.68% (single-strain) and 9.48% (mixed-strain) ($P<0.05$), with no significant difference between fermentation groups ($P>0.05$). Ether extract increased from 1.89% to 2.34% (single-strain) and 2.18% (mixed-strain) ($P<0.05$), again with no significant difference between groups ($P>0.05$).

2.5.2 pH Changes Table 11 shows that unfermented soybean meal had pH 6.42. Single-strain fermentation increased pH to 6.77 ($P>0.05$), while mixed-strain fermentation significantly decreased pH to 5.21 ($P<0.05$), which was also significantly lower than the single-strain group ($P<0.05$).

2.5.3 Soybean Globulin Content Changes Table 12 demonstrates that soybean globulin content decreased significantly after both fermentations ($P<0.05$), from 118 mg/g in unfermented meal to 56.12 mg/g (single-strain) and 70.22 mg/g (mixed-strain), with single-strain fermentation showing significantly greater reduction ($P<0.05$).

2.5.4 Protein Molecular Weight Changes Figure 9 [Figure 9: see original paper] shows that unfermented soybean meal contained substantial macromolecular proteins, primarily distributed at 35 and 45 kDa. After fermentation, proteins larger than 35 kDa decreased markedly, with distribution mainly below 25 kDa and minimally at 25 kDa. The single-strain fermentation group showed more proteins below 15 kDa compared to the mixed-strain group.

Discussion

3.1 Nutrient Content Changes After Fermentation

Microbial fermentation improved certain nutrient contents and effectively degraded anti-nutritional factors in soybean meal. In this study, small peptide content increased 10.61-fold and 8.68-fold after single-strain and mixed-strain fermentation, respectively, with single-strain fermentation producing significantly more peptides. Hong et al. [16] reported that fermentation increased <10 kDa peptide content, with unfermented meal containing 22.2% large peptides and

fermented meal containing no peptides >60 kDa, consistent with our findings. Elevated small peptide content results from microbial hydrolysis of proteins into amino acids, peptides, and ammonia [17-18]. The significant difference between fermentation groups likely occurred because protein-degrading enzymes primarily originated from *B. amyloliquefaciens*, which had relatively lower inoculation amount in mixed fermentation compared to single-strain fermentation.

Ma et al. [19] found that microbial fermentation increased crude protein content by 13.48%. Liu [20] reported that anaerobic fermentation by a *Bacillus subtilis* strain increased crude protein by 13.0% to 53.27%, while mixed fermentation by *B. subtilis*, yeast, and *Lactobacillus* increased it by 13.5% to 53.51%. Wang [21] observed crude protein reaching 59.52% after fermentation. In our study, crude protein increased by 18.44% (single-strain) and 20.21% (mixed-strain), with mixed-strain fermentation significantly higher than single-strain. Increased crude protein after solid-state fermentation primarily results from microbial respiration consuming organic matter and releasing CO and H O, causing "protein concentration effect" [22]. Additional protein originates from yeast cell protein and conversion of inorganic ammonium salts by yeast [23], representing the most meaningful contribution to crude protein increase. Microbial proliferation during fermentation not only elevated protein levels but also transformed plant proteins into microbial cell protein, improving protein quality.

Liu [24] found that compound microbial fermentation slightly increased crude protein but decreased crude fiber by 33.7%, while our study showed decreases of 26.84% (single-strain) and 26.05% (mixed-strain), possibly due to strain-specific degradation capabilities. Significant increases in ash and ether extract resulted from microbial utilization of organic matter causing dry matter loss and relative concentration, consistent with Fu [25] and Chi et al. [10].

3.2 pH Changes After Fermentation

Single-strain fermentation increased pH in our study, while Yang [26] observed significant pH reduction during *Lactobacillus* fermentation. This discrepancy likely arises because *B. amyloliquefaciens* produces proteases that degrade proteins into amines and ammonia, increasing pH, whereas *Lactobacillus* produces organic acids like lactic acid that decrease pH. Mixed-strain fermentation significantly reduced pH, possibly because aerobic *B. amyloliquefaciens* and *S. cerevisiae* created anaerobic conditions for *Lactobacillus* growth during early fermentation, while later *Lactobacillus* proliferation and lactic acid production neutralized amines and reduced pH, simultaneously improving flavor—an important reason for the acidic aroma of fermented feed [27], consistent with Liu [20] and Shi et al. [28].

3.3 Soybean Globulin Content Changes

Soybean globulin, the most heat-stable antigenic protein and primary cause of allergic reactions and diarrhea, was effectively degraded by fermentation. Fu

[25] reported 6.86-29.25% reduction using four different microorganisms, with mixed-strain fermentation showing similar effects to single strains. Our strains demonstrated higher degradation capacity, reducing soybean globulin by 52.65% (single-strain) and 40.75% (mixed-strain), with single-strain fermentation significantly more effective.

3.4 Protein Molecular Weight Changes

Li [29] found that unfermented soybean meal proteins concentrated at 45-66 kDa, with >20 kDa proteins largely degraded to <20 kDa after 72 h fermentation, showing gradual macromolecular protein reduction and micromolecular protein increase. Our study similarly showed unfermented proteins mainly >30 kDa, concentrated at 35 and 45 kDa, while fermented proteins were markedly shifted to <25 kDa, with complete degradation of >35 kDa proteins, concentrated at 15-20 kDa. Macromolecular protein degradation into smaller peptides and amino acids improves digestibility for animals while degrading antigenic proteins, enhancing protein quality and contributing to higher utilization efficiency of fermented soybean meal [11].

Conclusion

1. Growth curve analysis of *B. amyloliquefaciens*, *L. plantarum*, and *S. cerevisiae* determined the optimal inoculation time as 21 h.
2. Optimal conditions for single-strain solid-state fermentation of soybean meal by *B. amyloliquefaciens* were: 10% inoculation, 40°C temperature, 1.0:1.2 feed:water ratio, and 72 h fermentation time. Optimal conditions for mixed-strain fermentation were: 15% inoculation, 31°C temperature, 1.0:1.0 feed:water ratio, and 120 h fermentation time, with a strain ratio of *B. amyloliquefaciens*:*L. plantarum*:*S. cerevisiae* = 9:3:2.
3. Microbial solid-state fermentation increased small peptide content, improved nutritional value, degraded macromolecular proteins, and altered pH, with distinct differences between single-strain and mixed-strain fermentation effects.

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