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Effect of Inoculation with *Lacticaseibacillus casei* and *Staphylococcus carnosus* on the Quality of Squid (*Dosidicus gigas*) Surimi Sausage

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Abstract: *Dosidicus gigas* is a kind of low-fat food with poor gel properties. Fermentation has been proved to be an effective food processing method that could improve the gel properties of meat. Here, we inoculated *D. gigas* with two strains, *Lacticaseibacillus casei* and *Staphylococcus carnosus*, that have been approved for use in meat processing, and studied their impact on the quality of the product. Compared with the uninoculated samples, inoculation with *L. casei* and mixed inoculation with *L. casei* and *S. carnosus* were able to significantly reduce pH during fermentation. The plate counting results showed that *L. casei* may have adapted well to the environment in the inoculated groups, while the growth of *Staphylococcus* may have been inhibited in the mixed inoculated group. 16s rRNA sequencing confirmed that inoculation significantly altered the bacterial composition of squid surimi sausages. Both inoculation with *L. casei* and mixed inoculation with *L. casei* and *S. carnosus* were able to inhibit the accumulation of the main biogenic amines, and in the mixed inoculated group, the main biogenic amines were lower. Compared with unfermented squid surimi sausages, mixed inoculation changed the texture, gel properties, color, and appearance of squid surimi sausages. These results showed that mixed inoculation can not only ensure safety, but also improve the quality of squid surimi sausages.

Keywords: *Dosidicus gigas*; *Lacticaseibacillus casei*; *Staphylococcus carnosus*; sausage; texture; gel properties

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1. Introduction

Fermentation is an ancient and important food processing method that can not only improve the shelf life of products, but also their quality [1]. Fermented food is a good carrier of probiotics, but also contains bioactive substances that have been proven to be beneficial for the human body in many studies [2]. In China, many traditional foods are fermented, such as Cantone sausage, Sichuan sausage, and Jinhua ham [3]. With the development of the food industry, it has become necessary to use starter cultures in manufacturing fermented food in order to control the quality and safety of products [1].

In fermented meat products, the commonly used starters include lactic acid bacteria (LAB), coagulase-negative staphylococci (CNS), yeast, and mold [4]. LAB can inhibit the growth of other microorganisms by lowering pH and producing antibacterial substances [4]. Additionally, a decrease in pH can also induce changes in proteins, thereby improving the product's texture [5]. CNS are believed to be involved in improving the color and flavor of fermented meat products, mainly due to their rich enzymes, such as lipase, protease, nitrate reductase, catalase, and superoxide dismutase [5].

Dosidicus gigas is a kind of low-fat food with a high yield [6]. However, they will be frozen for a long time after capture, resulting in protein degradation and weak gel capability [7]. Many studies have confirmed that adding various ingredients to *D. gigas* can help improve gel properties. For example, adding the powder of *Porphyra haitanensis* to *D. gigas* surimi has been found to be an effective way to improve the breaking force,

water-holding capacity, and texture of surimi gel [8]. Adding sodium citrate and sodium tartrate to *D. gigas* also had similar effects [9].

Texture and gel strength are important quality parameters of surimi products [10]. There has been research confirming that inoculation could have positive effects on the texture and color of fish, like silver carp [11]; however, the effect of inoculation on the meat of Cephalopods, like *D. gigas*, has rarely been studied.

In the present research, we investigated the effects of inoculating with *L. casei* ATCC 393 and *S. carnosus* ATCC 51365, which have been previously used in the meat processing [12,13], on the quality of squid surimi sausages. The effect of inoculation on the pH, microbial counts, microbial community structure, biogenic amines, color, texture, and gel properties were studied. Additionally, the appearance of squid surimi sausages was also compared. This study evaluated the possibility of using *L. casei* and *S. carnosus* to produce fermented squid surimi sausages, which may contribute to the development of related products.

2. Materials and Methods

2.1. Materials

Dosidicus gigas were obtained from Ningbo Feirun Co. Ltd., Ningbo, China. The squid had had their skin previously removed and had been decapitated and cut into slices. Upon arrival, they were immediately stored below −20 °C

2.2. Preparation of Fermented Squid Surimi Sausages

L. casei ATCC393 and *S. carnosus* ATCC 51365 were obtained from Guangdong Microbial Culture Collection Center (GDMCC). These two bacteria were cultured three times at 37 °C for 24 h in de Man, Rogosa, and Sharpe (MRS) and nutrient broth (NB), respectively, and cells were harvested through centrifugation at 5000 rcf for 10 min at 4 °C (Beckman Coulter^{TM} Allegra 64R Centrifuge); then, the cell pellets were resuspended with a sterile 0.85% NaCl solution. After adjusting the cells to 10^9 CFU/mL (7.3×10^9 and 3.7×10^9 for *L. casei* and *S. carnosus*, respectively), the suspension was stored at 4 °C and used within the same day.

The frozen squid were thawed under running tap water and cut into 3 × 3 × 1 cm fillets. Then, the squid fillets were soaked in 2% sodium citrate (dissolved in cooled boiled water) in a ratio of 1:3 (*w*:*v*) for 15 h at 4 °C [14]. After washing three times, the squid were minced with a meat grinder. Then, 2% glucose, 3% NaCl, 8% corn starch, 3% soy protein isolates (corn starch and soy protein isolates were reported to improve the gel properties of the Cephalopods [6,15]), and 0.3% mixed phosphates and seasoning (the seasonings' ingredients were prepared as previously described for the Sichuan sausages [16]) were added to the sample. Starter cultures were inoculated to a final concentration of 10^7 CFU/g (7.3×10^7 and 7.4×10^7 for *L. casei* and *S. carnosus*, respectively) sample and mixed well. Three treatments of the squid surimi sausages were prepared as follows: (1) control (CK, no starter cultures were added), (2) LC (only *L. casei* added) and (3) LS (*L. casei* and *S. carnosus* added). The mixture was then stuffed into collagen casings (30 mm diameter). After punching holes on the casings with a sterilized toothpick, the sausages were fermented in an incubator (RLD-450E-4, Ningbo Ledian, Ningbo, China) at 30 °C RH 85% for 48 h.

2.3. Determination of pH

pH was measured as previously described with modifications [17]. Briefly, every 2 g of sausage was homogenized with 18 mL of cooled boiled water at 5000 rpm for 30 s (XHF-D, Ningbo Scientz, Ningbo, China), following which pH was measured using a pH meter (PB-10, Sartorius, Shanghai, China). pH determination of each group was performed in triplicate.

2.4. Microbiological Analysis

For this analysis, every 9 g of sausage was taken aseptically into a sterile homogeneous bag (BKman, Changde, China); then, 91 mL of a sterile 0.85% NaCl solution was added and

homogenized in a beating homogenizer (Jipad−20 cm, Shanghai Jipad, Shanghai, China) for 1 min. 1 mL sample was diluted with 9 mL of 0.85% NaCl to obtain a serial 10-fold solution. Following this step, 100 µL of an appropriate concentration of diluted solution was spread onto the respective agar medium in triplicate and incubated to determine the concentration of different microorganisms. Mannitol salt agar (MSA) agar was for *Staphylococcus* and incubated at 37 °C for 48 h, and MRS agar was for LAB and incubated anaerobically 37 °C for 48h. The results were expressed as $\log_{10}$ CFU/g.

2.5. DNA Extraction and Sequencing

2.5.1. DNA Extraction, PCR, and Libraries Construction

In this section, four samples for every group were collected and analyzed independently. After extracting total genomic DNA, the quality and quantity of DNA was analyzed using agarose gel electrophoresis; then, the DNA was diluted to 1 ng/µL as templates to amplify the V4–V5 region of the 16s rRNA gene using the following primers: F(GTGCCAGCMGCCGCGGTAA) and R(CCGTCAATTCCTTTGAGTTT) with barcodes. The PCR products were checked using agarose gel electrophoresis and then recovered. The libraries were constructed using the NEB Next® Ultra™ IIDNA Library Prep Kit, quantified with Qubit and qPCR, and then analyzed using NovaSeq6000.

2.5.2. Sequencing Data Processing and Analysis

To obtain Raw Tags, paired-end reads were assigned to samples based on barcodes and were truncated by cutting off the barcodes and primer sequences, then merged using FLASH (V1.2.11, http://ccb.jhu.edu/software/FLASH/, accessed on 9 October 2022) [18]. Subsequently, Clean Tags were obtained using fastp software (Version 0.20.0). Finally, chimera sequences were detected and removed using Vsearch software (Version 2.15.0) by comparing with the reference database (Silva database https://www.arb-silva.de, accessed on 9 October 2022) to obtain Effective Tags. Initial amplicon sequence variants (ASVs) were obtained using QIIME2 software (Version QIIME2-202006), following which the initial obtained ASVs were compared with the database to obtain species information for each ASV using QIIME2 software. Chao1, Simpson and Shannon indi-ces were obtained using QIIME2 software.

2.6. Determination of Biogenic Amines

The extraction of biogenic amines was performed in accordance with the China national standard method (GB 5009.208—2016) with minor modifications. The squid sausage samples were accurately weighed and then transferred to centrifuge tubes and homogenized with 5% trichloroacetic acid (TCA) at 5000 rpm for 1 min. After shaking for 30 min in an incubator (TS-UR, Zhejiang Huayuan, Hangzhou, China) and centrifuging 5000 rpm for 10 min, the supernatant was collected in a 25 mL volumetric flask and the residue was extracted again as described above. The volume of the supernatant was made up to 25 mL with 5% TCA. BAs were derivatized using a previously established method with modifications [19]. The volume of dansyl chloride (10 mg/mL) was 1.5 mL, and the reaction time was 35 min. HPLC analysis was performed using an Agilent 1260 system (Agilent Technologies, Paolo Alto, CA, USA). The gradient elution program was performed through changing the proportion of water and acetonitrile as previously described [20]. The flow rate was set as 1 mL/min and the column temperature was set to 40 °C. The injection volume was 20 µL. The spectra were acquired at 254 nm. The concentration of BAs was determined in triplicate.

2.7. Texture Profile Analyses, and Rupture and Color Tests

To perform texture profile analyses, rupture and color test of the squid surimi sausages, the samples were firstly heated in 40 °C for 60 min and 90 °C for 30 min in a water bath, and then immediately cooled in cooled boiling water for 30 min and stored at 4 °C for further analysis.

Texture profile analysis (TPA) was performed using a TA. XT Plus texture analyzer (Stable Micro System, Godalming, UK) with a P/50 probe (50 mm diameter). The squid surimi sausages were cut into 20 mm in length. After removing their casings, TPA was conducted with the following parameters: pre-test speed, 1 mm/s; test-speed, 1 mm/s; post-test speed, 5 mm/s; target model, strain; strain, 30%; time, 5 s; and trigger force, 5 g. TPA were performed 7 replicates per group. During the TPA test, one sample in the LS group (inoculated with *L. casei* and *S. carnosus* and fermented for 48 hours) was aborted due to overloading, so in LS group, TPA data were collected and analyzed with only 6 samples.

The rupture test was performed using the same texture analyzer (TA. XT Plus, Stable Micro System, Godalming, UK) with a P/5 probe (5 mm diameter). The parameters were as follows: test mode, compression; pre-test speed, 1.5 mm/sec; test speed, 1 mm/sec; post-test speed, 1 mm/sec; target mode, distance; distance, 10 mm; and trigger force, 5 g. The rupture strength was defined as the force of the first peak during the rupture process, while the rupture hardness was defined as the force of the maximum peak during the test according to the manufacturer's instructions. Rupture tests were performed 7 replicates for each kind of sample.

The color of the samples was determined through measuring the values of L* (lightness), a* (redness), and b* (yellowness) of 3 different areas of the same sample using a color meter (NH310, 3NH, Shenzhen, China) equipped with D65 as the illuminant source. The color tests were measured 6 replicates per group.

2.8. Appearance Photograph

The casings of the squid surimi sausages were removed, following which the samples were placed on white paper and photographed using a smartphone (IQOO U3X, ViVo, Dongguan, China). To get the appearance of the cross-sectional view, the samples of different groups were cut into identical lengths, and then photographed as abovementioned.

2.9. Statistical Analysis

One-way analysis of variance and the Student's *t*-test were performed using IBM SPSS software. Statistically significant differences were defined at $p < 0.05$.

3. Results and Discussion

3.1. Effect of Inoculation on pH of the Squid Surimi Sausages

According to the announcement issued by the National Health Commission of China in 2022, both *L. casei* and *S. carnosus* were approved to be used in food processing. Specially, the two strains *L. casei* ATCC 393 and *S. carnosus* ATCC 51365 both had records in food processing [12,13]; therefore, we did not evaluate the safety of these two strains in the present study.

For fermented sausages, the rapid decrease in pH is crucial for the safety of the product, as a low pH environment can inhibit the growth of unwanted microorganisms [21]. Figure 1 shows pH changes in the different groups during the fermentation process from 0 h to 48 h. As the fermentation proceeded, pH of all three groups decreased. At 48 h, pH of the CK group (control, non-inoculated group) was significantly higher than that of the LC (inoculated with *L. casei*) and LS (inoculated with *L. casei* and *S. carnosus*) ($p < 0.001$) groups, while there was no significant difference found between the LC and LS groups ($p > 0.05$). This decrease in pH may be due to the organic acids produced by LAB, and previous studies have confirmed that the amounts of several organic acids were increased during the fish fermentation [22]. Therefore, for squid surimi sausages, the CK group may have a higher safety risk, while LC or LS can lower pH and ensure safety. These results were similar with findings in dry-cured foal sausage [23], dry fermented mutton sausages [24] and Chinese Cantonese sausages [25], which were performed by other authors who reported that pH in inoculated sausage was lower compared to non-inoculated ones.

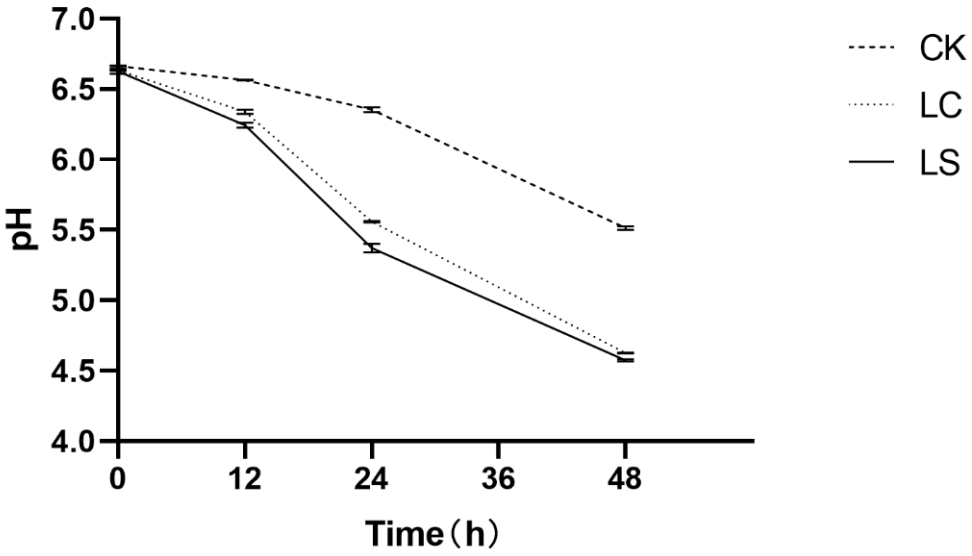

Figure 1. pH change during the fermentation process. CK represents the non-inoculated group, LC represents the group inoculated with *L. casei*, and LS denotes the group inoculated with *L. casei* and *S. carnosus*.

3.2. LAB and Staphylococcus Counts during Fermentation

Figure 2 displays the changes of LAB and *Staphylococcus* during the fermentation process in different groups. It can be easily concluded that LAB in all groups gradually increased within 48 h, and that the growth of LAB in the LC and LS groups was slower than that observed in the CK group (Figure 2a). At 0 h, the counts of LAB in the CK group were lower than those in the LC and LS groups, due to LAB in CK at this period were from the raw material, indicating that LAB in the CK group were on a relatively small scale. At 48 h, LAB in the CK group were still significantly lower than that of LC and LS groups ($p < 0.001$). *Staphylococcus* numbers also increased within 48 h in the CK and LC groups (Figure 2b). At 24 h, the counts of *Staphylococcus* in the CK group were significantly higher than that of the LC group ($p < 0.001$), probably due to *Staphylococcus* in the LC group was inhibited by LAB. Between 24 h to 48 h, the growth of *Staphylococcus* in the CK and LC groups became slower, and the number of *Staphylococcus* in the LS group showed a slight decrease at the same period, which may have been due to their poor competitiveness against LAB [26]. Though the growth of *Staphylococcus* in the LS group was not fast as that of the CK and LC groups, the number of *Staphylococcus* in the LS group were always higher than those of CK and LC groups. These results suggest that *L. casei* may be well adapted to the environment, and that they can grow and maintain in squid surimi sausages. *S. carnosus* in the LS group grew slowly and even decreased between 24 h and 48 h; however, they were still observed at a high level, which might benefit the quality improvement of squid surimi sausages due to their rich enzymes [5].

3.3. Effect of Inoculation on the Bacterial Community of the Squid Surimi Sausages

The plate counting method cannot distinguish the microorganism at the genus level. Therefore, we performed 16S rRNA sequencing to study the effect of inoculation on the bacterial community composition of squid surimi sausages.

Good's coverage is an index that can be used to evaluate the sequencing coverage [27]. The results (as displayed in Table 1) revealed that the Good's coverage rate of all samples reached the value of one, indicating that most bacteria in the samples were covered with the present sequencing strategy. Additionally, rarefaction curves can also reflect the sequencing coverage [28]. Figure 3a supported that the sequencing data were sufficient to analyze the bacterial composition.

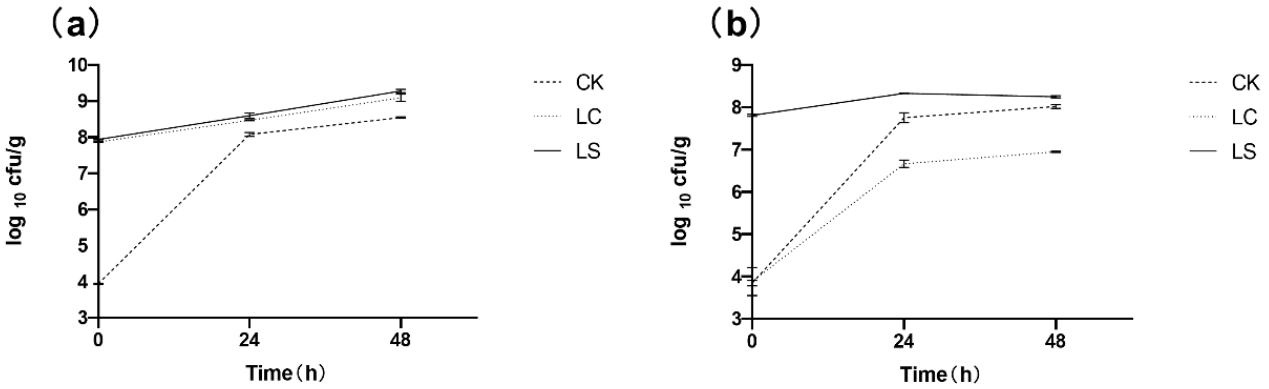

Figure 2. Counting LAB (**a**) and *Staphylococcus* (**b**) during the fermentation process. The meanings of CK, LC, and LS are consistent with that outlined in Figure 1.

Table 1. Diversity analysis of V4–V5 region of the 16s RNA in different fermented squid surimi sausages.

Sample	Chao1	Shannon Index	Simpson Index	Good's Coverage
CK1	144	3.971	0.851	1
CK2	160	3.458	0.76	1
CK3	141	3.657	0.83	1
CK4	161	4.045	0.869	1
LC1	89	1.617	0.395	1
LC2	76	1.522	0.383	1
LC3	84	1.787	0.435	1
LC4	97	1.811	0.439	1
LS1	58	1.308	0.327	1
LS2	80	1.421	0.343	1
LS3	71	1.496	0.364	1
LS4	62	1.523	0.371	1

CK represents the non-inoculated samples. LC denotes the fermented samples inoculated with *L. casei*. LS represents the fermented samples inoculated with *L. casei* and *S. carnosus*. All these sample were fermented for 48 h.

Alpha diversity can be evaluated using several indices, including the Chao1, Shannon, and Simpson indices [29]. Chao1 is used to evaluate richness, while Simpson and Shannon indices are used to calculate the diversity of the microbial community [30]. CK samples exhibited a higher richness compared to the LC and LS groups, evidenced by the higher Chao1 in the CK group (Table 1). Furthermore, the Shannon index and Simpson index were higher for the CK group when compared to those obtained for the LC and LS groups, suggesting a higher diversity for the CK samples (Table 1).

The bacterial composition of the three groups at 48 h are presented in Figure 3b,c. Figure 3b displays the bacterial composition at the phylum level. Four kinds of phyla were identified in these samples. In the CK samples, the most abundant phyla were Proteobacteria and Firmicutes. However, in the LC and LS samples, the proportion of Proteobacteria decreased, and Firmicutes was the most abundant. The bacterial composition of these three groups at the genus level are shown in Figure 3c. It is clear that the bacterial composition of the CK sample was significantly different from that of the LC and LS samples. In the CK group, the two most abundant genera of bacteria were *Streptococcus* and *Lactococcus*, accounting for 36.7% and 18.5%, respectively. It has been reported that many bacteria in *Streptococcus* genus are able to produce biogenic amines (BAs) [31]. In the LC and LS groups, the proportion of these two bacteria significantly decreased. The highest proportion of bacteria for both the LC and LS groups was *Lactobacillus*, accounting for approximately 89.6% and 94.2%, respectively, while in the CK group, its proportion was around 2.6%. As for *Staphylococcus*, the proportions were approximately 0.37%, 0.14%,

and 1.1% in the CK, LC, and LS groups, respectively. Consistent with the plate counting result, the *Staphylococcus* proportion of the LC group was lower than that of the CK group, possibly due to the inhibition of LAB. Similar findings have also been found in previous research [32], which revealed that *Staphylococcus* in LAB-inoculated samples was lower than that of the non-inoculated samples. The percentage of *Staphylococcus* in the LS group was higher than that of the CK group, indicating that *S. carnosus* could grow or maintain in squid surimi sausages. CNS have been believed to contribute to the color and flavor development of sausage [5], but the proportion of CNS in the LS group were relatively low, indicating that they may have limited contribution to color and flavor of the product, and further research is needed. In addition, for bacteria identified as others, for the CK group the proportion was close to 32%, while for the LC and LS groups the proportions were very low, with values of about 2.3% and 1.1%, respectively, highlighting the necessary of inoculation in fermented squid surimi sausages. In summary, the bacterial compositions of squid surimi sausages were significantly altered in the LC and LS groups, with *Lactobacillus* dominant. These results are in agreement with study reported by other authors, who found that inoculation with *Lactobacillus plantarum* R2 or co-inoculated with *L. Plantarum* R2 and *S. xylosus* A2 in Chinese dry fermented sausages could affect the bacterial composition [33]. LAB, including *Lactobacillus*, can inhibit the growth of other bacteria through producing organic acids, such as lactic acid, acetic acid, formic acid, and propionic acid, which have antibacterial effects, and other antibacterial substances, such as hydrogen peroxide and bacteriocins, and might benefit the quality and safety of the meat products [4]. Although the percentage of *Staphylococcus* in the mixed inoculated group was low, they might still maintain at a high level when considering the result of plate counting, implying that they might be involved in the quality development of the samples.

3.4. Effect of Inoculation on the BAs Accumulation of Squid Surimi Sausages

BAs are mostly formed through the decarboxylation of amino acids [34], and the formation of BAs is related to amino acids along with some microorganisms [35]. BAs have been proven to be involved in several biological functions; however, the intake of a high concentration of BAs may impose health concerns, especially to the susceptible individuals [34]. Additionally, BAs can be also be converted into nitrosamine [35]. Therefore, the concentration of BAs in fermented food is a concerned issue. In the present study, eight kinds of BAs were measured, including tryptamine (TRY), phenethylamine (PHE), putrescine (PUT), cadaverine (CAD), histamine (HIS), tyramine (TYR), spermidine (SPD), and spermine (SPM).

The results displayed in Figure 4 indicate that only a small amount of SPM was detected in the unfermented squid surimi sausages. After 48 h of fermentation, the total amount of BAs in all three groups were increased compared to the unfermented samples. Specifically, the total BAs in the CK group was about 2.6 times and 3.2 times higher than that of the LC and LS groups, respectively. This was mainly due to the higher concentration of TYR, PUT, CAD, and HIS in the CK group. In the non-inoculated samples, the most abundant BA was PUT, followed by CAD, HIS, and TYR. In the LC and LS samples, TYR was the most concentrated BA, followed by CAD, PUT, and HIS. Statistical analyses showed that the concentration of PUT and CAD in the CK group were significantly higher than those of the LC and LS groups ($p < 0.05$); meanwhile, PUT and CAD in the LC group were significantly higher than those observed in the LS group ($p < 0.05$). The concentration of HIS in the CK group was significantly higher than that of the LC and LS groups ($p < 0.05$). The concentration of TYR in the group CK was higher than that of the LC and LS groups, and TYR in the LC group was higher than that observed in the LS group ($p < 0.05$).

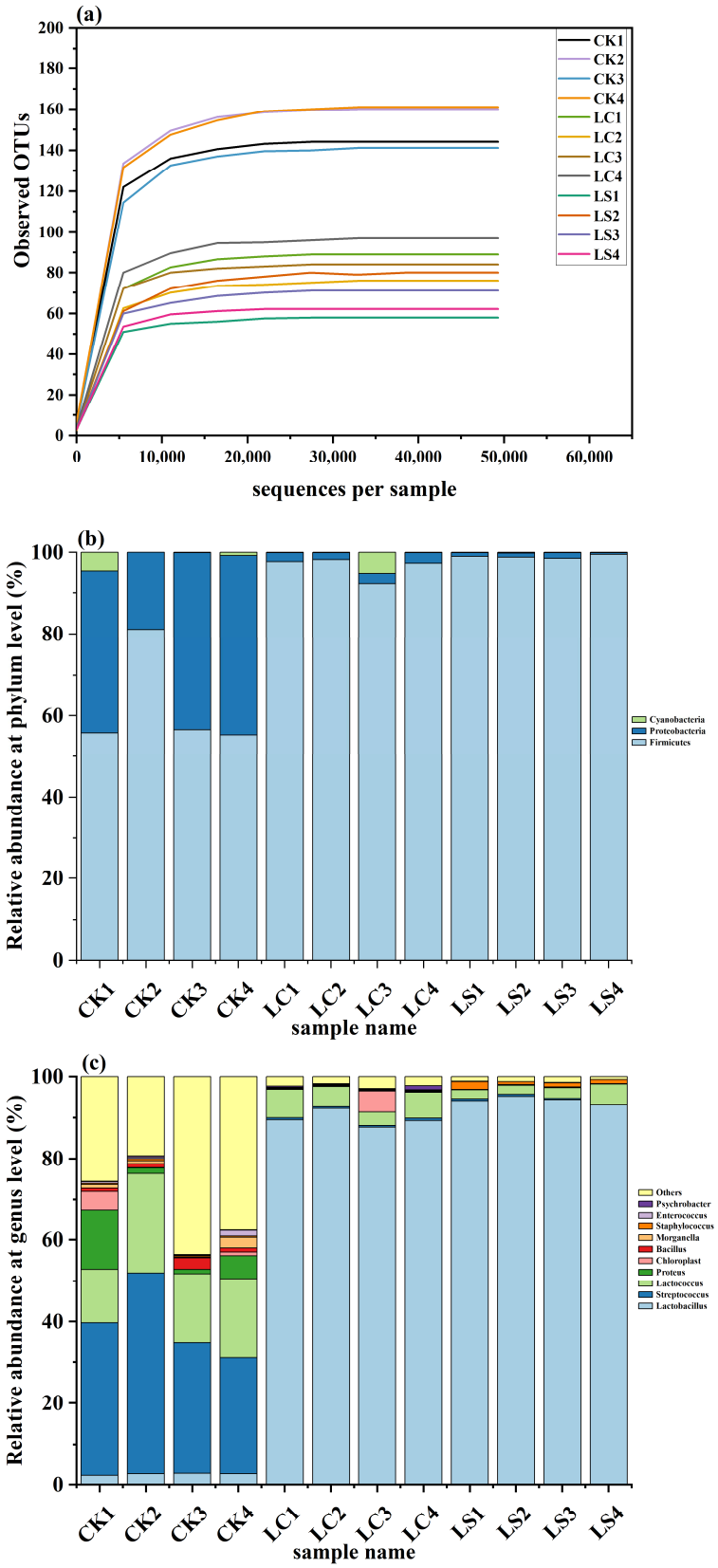

Figure 3. Rarefaction curves (**a**) and bacterial composition at the phylum (**b**) and genus (**c**) level of different fermented squid surimi sausages. CK represents non-inoculated samples. LC denotes the fermented samples inoculated with *L. casei.* LS represents the fermented samples inoculated with *L. casei* and *S. carnosus.* All these samples were fermented for 48 h.

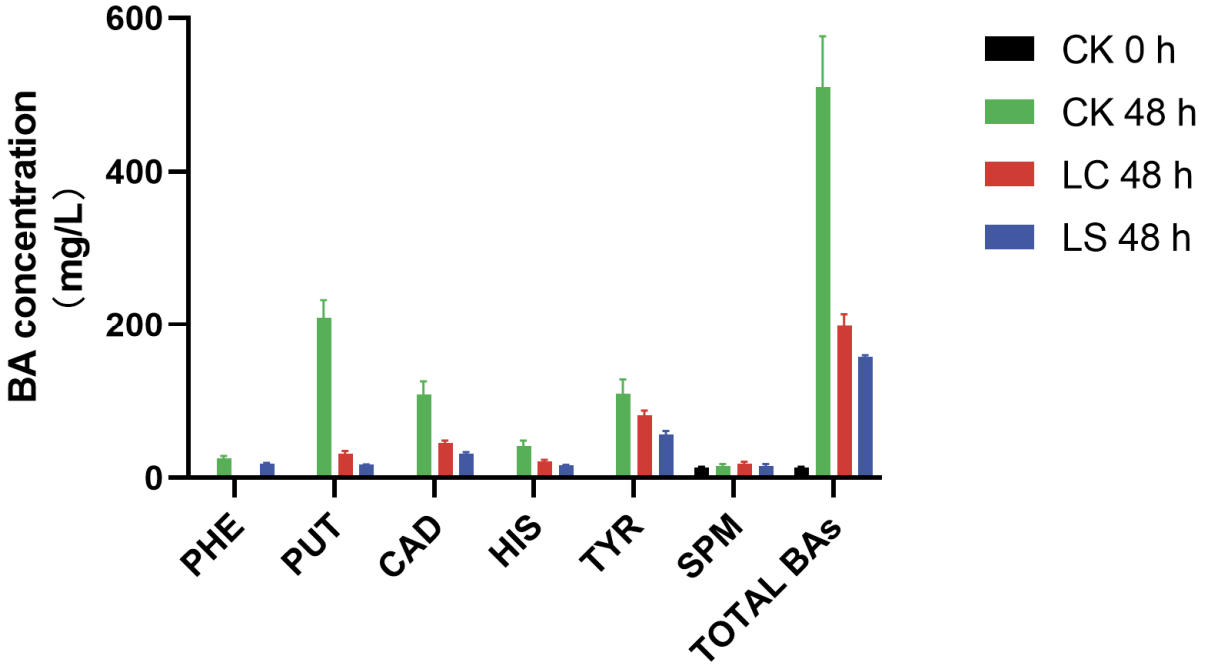

Figure 4. Biogenic amines of the squid surimi sausages. CK 0 h, CK 48 h, LC 48 h, and LS 48 h are for non-fermented, non-inoculated and fermented for 48 h, inoculated with *L. casei* and fermented for 48 h, and mixed inoculated with *L. casei* and *S. carnosus* and fermented for 48 h groups, respectively.

HIS and TYR are considered the most toxic biogenic amines [36]. PUT and CAD cannot cause direct health concerns; however, they can improve the toxicity of HIS, and they can also be transformed into nitrosamines [37]. SPM was detected in the unfermented sample, CK, LC, and LS groups; however, there was no significant difference between the four groups ($p > 0.05$). This may be because SPM was mainly from the raw materials, and microorganisms cannot produce it through the decarboxylation of amino acids; meat, microorganisms can use it as a nitrogen source [38]. Although PHE was detected in the CK and LS groups, its content was relatively low. Therefore, PHE was also not the main BA in fermented squid surimi sausages. In conclusion, inoculation could inhibit the formation of the four main BAs (HIS, TYR, PUT, and CAD), while co-inoculation could further reduce the amount of these four kinds of BAs, demonstrating that *L. casei* and *S. carnosus* can potentially be used as starter culture to improve the safety of fermented squid surimi sausages. From the perspective of reducing the content of BAs, mixed inoculation was a better choice than using *L. casei* alone.

Consistent with our results, there have been many findings reporting that inoculated fermented meat had low concentrations of BAs. For instance, inoculation with *Pediococcus pentosaceus*, *L. sakei*, *S. xylosus*, and *S. carnosus* to Sichuan sausages could inhibit the increase in HIS, PUT, CAD, and TYR [16]. Similar results have also been found in fermented fish (Suanyu) [39].

3.5. Effect of Inoculation on the Texture, Rupture Strength, and Color of Squid Surimi Sausages

Texture profile analysis (TPA), a widely used method to determine the texture properties of food, includes several parameters, like hardness, springiness, cohesiveness, and chewiness [40]. The changes in hardness, springiness, cohesiveness, and chewiness of the unfermented and mixed inoculated squid surimi sausages are presented in Table 2. It can be concluded that after 48 h of fermentation, the hardness ($p < 0.01$) and chewiness ($p < 0.01$) of the samples significantly increased, while the springiness ($p < 0.01$) and cohesiveness ($p < 0.01$) decreased, compared to the unfermented samples. These results were consistent with previous findings regarding fermented tilapia sausage, which reported that inocu-

lation of *P. pentosaceus* 30-7 into tilapia sausage were able to improve the hardness and chewiness parameters, while reducing the springiness and cohesiveness, when compared with the unfermented sample [31]. The rupture strength ($p < 0.01$) and hardness ($p < 0.01$) were also significantly higher than those of the unfermented samples (Table 2), consistent with the TPA test. The changes in the TPA and rupture test may be attributed to a decrease in pH, as decrease in pH can lead to protein aggregation and the formation of ordered networks, thus leading to an increase in firmness [41]. The texture characteristics of sausage have also been associated with water addition in starch-meat sausage [42]. The changes in these TPA and rupture parameters of the squid surimi sausages may be the combined effect of pH and moisture. In addition, during the processing of golden pompano surimi, springiness and cohesiveness were found to be positively correlated with the water-holding capacity (WHC) [43], implying that fermented squid surimi sausages may have a lower WHC after cooking when compared to the unfermented samples, and further research is therefore needed.

Table 2. TPA, rupture test, and color test of squid surimi squid sausages.

	CK	**LS**
TPA test		
Hardness (g)	1893.086 ± 194.019 [a]	4219.882 ± 269.484 [b]
Springiness	0.948 ± 0.039 [a]	0.849 ± 0.019 [b]
Cohesivenes	0.799 ± 0.007 [a]	0.686 ± 0.040 [b]
Chewiness (g)	1432.319 ± 139.941 [a]	2457.859 ± 248.508 [b]
Rupture test		
Rupture strength (g)	178.071 ± 16.030 [a]	355.209 ± 82.139 [b]
Rupture hardness (g)	186.528 ± 17.234 [a]	371.631 ± 92.166 [b]
Color test		
L	49.475 ± 0.289 [a]	55.320 ± 1.726 [b]
a	11.883 ± 0.493 [a]	15.003 ± 0.408 [b]
b	19.488 ± 0.330 [a]	23.752 ± 0.618 [b]

CK represents the non-fermented samples, while LS represents the mixed inoculated samples with *L. casei* and *S. carnosus* and fermented for 48 h. Prior to the test, the samples were heated as described in the Materials and Methods sections. Different lowercase letters indicate a significant difference between the different samples.

Color is an important property of food that might influence consumers' purchase behavior [44]. As shown in Table 2, the L ($p < 0.01$), a ($p < 0.01$), and b ($p < 0.01$) parameters of the LS group were significantly higher than those of the unfermented samples, indicating that the brightness, redness, and yellowness of the LS group were higher than those of the unfermented samples. From the naked eye, the brightness of the LS group was higher than that of the unfermented samples (Figure 5), while the difference in the redness and yellowness were not found to be significantly different, which may be due to the insufficient difference of a and b between the two groups. In pork, the color of the meat products was related to the content and state of myoglobin [45]. In addition, some studies suggested that protein degradation and oxidation could affect the color of meat products, as protein degradation and oxidation could change the microstructure of proteins, thus affecting the propagation of light and the color of the meat [45]. Additionally, water addition can also influence the color of starch-meat sausage [42], suggesting that the moisture of squid surimi sausages after cooking might also influence their color.

Additionally, unfermented squid surimi sausages were prone to bending after cooking, while fermented sausages were relatively uniform in shape (Figure 6), which may be more favored by consumers.

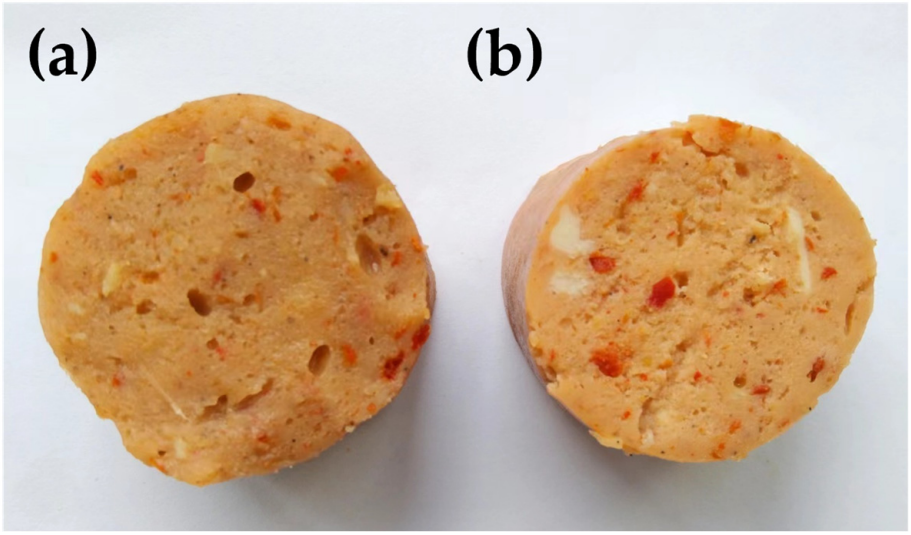

Figure 5. Typical appearance of an unfermented squid surimi sausages (**a**) and a mixed inoculated squid surimi sausages with *L. casei* and *S. carnosus* (**b**) after cooking (cross-sectional view). The cooking method was described in the Materials and Methods section.

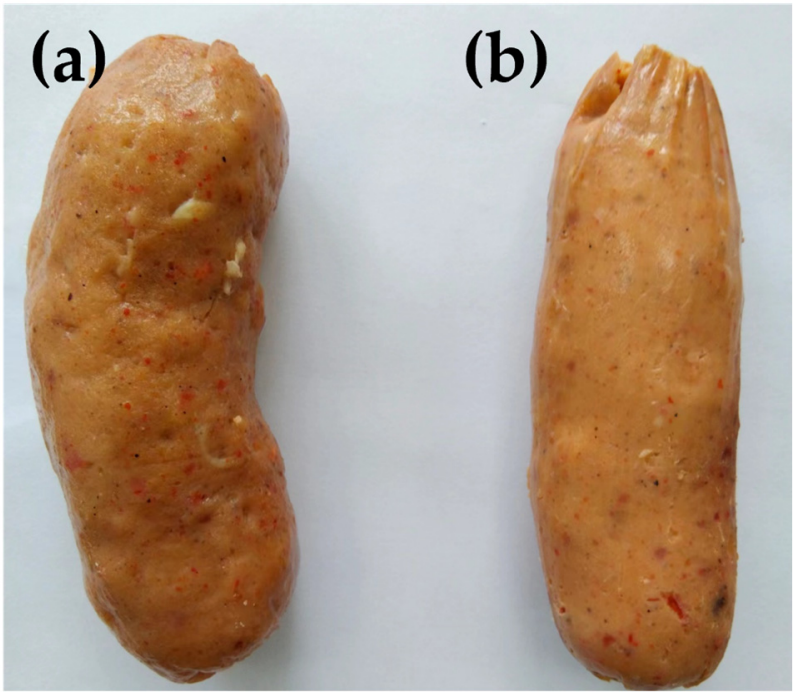

Figure 6. Typical appearance of an unfermented squid surimi sausages (**a**) and a mixed inoculated squid surimi sausages with *L. casei* and *S. carnosus* (**b**) after cooking (casings removed). The cooking method was outlined in the Materials and Methods section.

In summary, compared with unfermented squid surimi sausages, the mixed inoculated samples had higher hardness, chewiness, rupture strength, rupture hardness, and brightness, and their shapes were also maintained.

4. Conclusions

In the present study, we confirmed that co-inoculation of *L. casei* ATCC393 and *S. carnosus* ATCC 51365, which have been previously used in meat processing, could ensure safety, and also improve texture properties, gel properties, color, and appearance of

the squid surimi sausages. Our results suggest that mixed inoculation with *L. casei* and *S. carnosus* may be a possible method to improve the quality of squid surimi sausages. However, further research is needed to clarify the underlying mechanism.

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