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Effects of *Lactobacillus sakei* inoculation on biogenic amines reduction and nitrite depletion of chili sauce

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Abstract

In this study, the effects of the starter culture *Lactobacillus sakei* on pH, the total lactic acid concentration, nitrite depletion and histamine reduction of chili sauce were investigated. The nitrite concentration in the CPN sample was significantly lower ($p < 0.05$) than that of the CPS sample (as control), and decreased by 30.87%.The putrescine and histamine concentration in starter culture inoculated chili sauce were significantly lower ($p < 0.05$) than that of the control sample, and were decreased by 38.08% and 49.88%, respectively. These results revealed that *L. sakei* could be used as a potential starter culture for nitrite depletion and biogenic amines reduction of chili sauce with good sensory attributes.

Keywords: biogenic amines; chili sauce; *Lactobacillus sakei*; nitrite depletion.

Practical Application: Chili sauce as a Chinese typical fermented condiment product is well preferred by most Chinese consumers. However, the hazards of harmful substances in fermented foods had attracted researchers to attention. The *Lactobacillus sakei* was often added to fermented sausage as a biological agent to control the levels of nitrite and biogenic amine in fermented foods. In order to investigate the effects of starter culture on harmful substances of chili sauce, we systematically screened *Lactobacillus sakei* as starter culture. The results showed that starter culture could decrease nitrite and putrescine and histamine concentration of chili sauce. These results provide valuable information for application of *Lactobacillus sakei* in chili sauce.

1 Introduction

Chili sauce as the Chinese traditional fermented condiment has been well preferred by Chinese consumers, especially in Sichuan province in China, due to its delicious flavor and strong characteristic of appetite enhancement. Chili sauce is usually composed with chili, garlic, salt, and sucrose, and manufactured by spontaneous fermentation or starter culture inoculation. The deterioration in sensory properties of chili sauce by spontaneous fermentation, including flavor, color, and texture, are mainly caused by foodborne spoilage microorganisms and pathogenic bacteria, resulting in the chili sauce that is not homogenous or one that is of inferior quality with safety standards that cannot be guaranteed. It has been reported that the gas-forming spoilage bacteria, mainly identified as *Bacillus licheniformis, Lactobacillus acidipiscis,* and *Lactobacillus alimentarius*, have been isolated from chili sauce (Niu et al., 2020). Furthermore, pathogenic bacteria, such as *Escherichia coli*, *Yersinia enterocolitica,* and *Bacillus thuringiensis*, have been detected in chili sauce (Niu et al., 2020; Estrada-Garcia et al., 2002). Thus, microbial quality during fermentation is one of the key factors in determining the sensory, flavor, and safety profiles of chili sauce.

Recently, the starter cultures have been applied into fermented foods to improve their nutritional quality and prolong shelf life. These starter cultures could induce the production of bacteriocin or acidic byproducts, which could inhibit the growth of foodborne pathogens (Kim et al., 2018; Syahbanu et al., 2020). It is noteworthy that *L. sakei* can thrive in high-salt, low-water, low-temperature, and low pH conditions (Chiaramonte et al., 2009), and has an ability to produce bacteriocins to inhibit pathogenic bacteria (Aasen et al., 2000). It has been reported that *L. sakei* inoculation in fermented food could inhibit the growth of Enterobacteriaceae and *Listeria monocytogenes* (Wang et al., 2015; Wang et al., 2016).

Moreover, various biogenic amines, such as histamine and putrescine, have been detected in Chili sauce (Wunderlichová et al., 2014), resulting in an increasing attention among consumers. The formation of biogenic amines is mainly attributed to the presence of decarboxylase-producing microorganisms, which may be introduced by contamination in Chili sauce (Świder et al., 2020). Thus, the growth of the potential biogenic amines producers should be controlled. It has been reported that certain starter culture inoculation was conducive to inhibiting the growth of biogenic amines producers, resulting in a decrease in biogenic amines levels in fermented food (Wang et al., 2021; Jia et al., 2020).

Although the value of the starter culture is well known, its contribution to nitrite depletion and biogenic amine reduction in Chili sauce has not been fully described. Therefore, it is interest to further characterize nitrite depletion and biogenic

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amines decrease of Chili sauce fermented by the starter culture inoculation. In this study, the starter culture of *L. sakei* was inoculated in chili sauce. Comparative analysis of the contribution to decrease in biogenic amines levels and nitrite depletion in chili sauce between starter culture inoculation and spontaneous fermentation were performed to gain insight regarding the improvement of food safety.

2 Materials and methods

2.1 Starter culture

L. sakei as a starter culture used to manufacture chili sauce was supplied by the Meat Processing Application Key Lab of Sichuan Province in China. *L. sakei* was cultured to a final concentration of 10^7–10^8 CFU/g and then immediately freeze-dried for use as a starter culture in subsequent experiments.

2.2 Chili sauce preparation and sampling

The recipe for chili sauce was employed as the recipe described by Niu et al. (2020) with a minor modification. Chili sauce was manufactured according to the following recipe to yield a kilogram of the final product: chili (760 g), salt (60 g), garlic (80 g), Chinese prickly ash (3 g), Allium macrostemon Bunge (25 g), Litsea pungens Hemsl (50 g), and other ingredients (20 g). The chilies were crushed and mixed with the other ingredients, and the resultant mixture was inoculated with 0.05% (w/w) of the starter culture. The batch with the starter culture inoculation was labeled as CPN, and the sample without the starter culture inoculation was labeled as CPS (as the control). After mixing, the mixture was transferred into 5 liter ceramic jars and fermented anaerobically at 25 °C with a humidity level of 85-90%. The chili sauce (100 g) was periodically sampled in triplicate from fermentation on day 0, 5, 10, 15, 20, 25, 30, 35, and 40 for HTS, physicochemical properties analyses and biogenic amine concentration measurement.

2.3 pH and total lactic acid concentration measurement

At each time point, the pH value of chili sauce was measured by a pH meter (Testo 205, Testo International Trade Co., Ltd., Shenzhen, China) with automatic temperature compensation (NTC) electrode according to the method described by Wang et al. (2018a). Before measurement, the pH probe was calibrated using buffers at pH 4.00 and 7.00 at room temperature. Inserting the electrode directly into tested samples, triplicate readings were obtained from three different random locations and then averaged.

The total lactic acid concentration of chili sauce was determined by using a commercial kit D-/L-lactic acid enzymatic bioanalysis (Boehringer Mannheim/R-Biopharm AG, Darmstadt, Germany) as described by Wang et al. (2019), which is based on the reaction catalyzed by lactate dehydrogenase and glutamate pyruvate transaminase.

2.4 Nitrite concentration measurement

The nitrite content in chili sauce was determined according to colorimetric nitrite assay based on the Griess reaction as described by Ito et al. (1979). The 5.0 g chili sauce was sampled. Then 12.5 mL borax saturated solution was added and transferred into a 500 mL volume flask. After ultrasonic extraction for 30 min, it was shaken once every 5 min. After cold extraction, 5 mL potassium ferrocyanide was added. After shaking, 5 mL zinc acetate was added. Finally, the volume was fixed to 500 mL. 30 mL of the initial filtrate was discarded and 40 mL of the filtrate was transferred into a 50 mL colorimetric tube with stopper. 2 mL of p-aminobenzene sulfonic acid solution (4 g/L) was successively added into the sample tube. After mixing, 1 mL of naphthalene ethylenediamine hydrochloride solution (2 g/L) was successively added and water was added to the 50 mL scale line. The absorbance value was measured at 538 nm. Each test was repeated three times. The nitrite content was expressed as mg/kg of sample.

2.5 Putrescine and histamine concentration measurement

The putrescine and histamine concentration was measured by high performance liquid chromatography (HPLC) as described by Lu et al. (2010) and Gaviao et al. (2021). 5 g chili sauce was homogenized in 20 mL 5% trichloroacetic acid and shaken for 30 min. Then the mixture was centrifuged at 5000 r/min for 10 min. The supernatant was filtered and transferred into a 50 mL volumetric flask. The residue was extracted twice with n-butanol/chloroform. Finally, the extraction was measured with 5% trichloroacetic acid to 50 mL for HPLC analysis (Agilent 1100 Series, Agilent Technologies Inc., Palo Alto, CA). The separation was performed on a Hypersil BDS C18 (4 × 125 mm) column (Thermo Fisher Scientific, Waltham, MA). The gradient program started with 60% acetonitrile and 40% ammonium acetate and then increased the acetonitrile to 100% in 25 min. Then acetonitrile was reduced to 60% from 32 to 37 min. The column temperature was 35 °C and flow rate was 0.8 mL/min. Biogenic amines were detected at 254 nm. A standard containing putrescine and histamine is used as an internal standard. Standard putrescine and histamine was purchased from Sigma (San Francisco, CA). Each test was repeated three times. The putrescine and histamine content was expressed as mg/kg of sample.

2.6 Sensory evaluation

The sensory evaluation of chili sauce was performed by 31 sensory evaluation personnel after the CPS and CPN samples were numbered (Boeno et al., 2019). All samples were coded using three-digit numbers and presented to the 31 assessors in random order. The color, flavor, status, and taste were evaluated by assessors using the hedonic scale ranging from 1 (dislike extremely) to 25 (like extremely) as shown in Supplemental Table 1.

2.7 Statistical analysis

Each test was performed in triplicate and these results were reported by means with standard deviation. Data were displayed as mean values accompanied with the standard deviation. Duncan's multiple range test (significance was defined at $p < 0.05$) was employed for the independence of error terms using the SPSS statistics software (IBM, Chicago, Ill., U.S.A.).

3 Results and discussion

3.1 Effect of the starter culture on physical properties in chili sauce

The effects of *L. sakei* on pH and total lactic acid concentration of chili sauce are shown in Figure 1. The initial pH value was 5.21 and 5.24 in the CPS and CPN samples, respectively. During fermentation, a decrease in pH was detected in CPS and CPN samples, and the decrease was more intense in CPN sample ($p < 0.05$), probably due to the lactic acid accumulation derived from *L. sakei* which was inoculated in CPN sample (Figure 1a). Specifically, the pH in the CPS sample decreased from 5.21 to 3.81 on the 40th day. In contrast, the pH in CPN sample decreased rapidly from 5.24 to 3.80 on the 25th day. These results showed that the pH in the chili sauce fermented by *L. sakei* was significantly lower than that of spontaneous fermentation as shown in Figure 1a ($p < 0.01$).

The changes in the total lactic acid level in chili sauce during fermentation are shown in Figure 1b. The total lactic acid in the chili sauce inoculated with *L. sakei* was higher than that of spontaneous fermentation during the entire fermentation. The total lactic acid increased from the initial value of 4.52 mg/kg to 8.27 mg/kg in the CPN sample and from the initial 4.53 mg/kg to 7.86 mg/kg in the CPS sample, respectively. On the 40th day, the total lactic acid content of CPN sample was 8.27 mg/kg, which was significantly higher than that of the CPS sample as shown in Figure 1b ($p < 0.01$). It has been reported that *L. sakei* is capable of producing high levels of organic acids and lowering the pH to below 4.0, which may be the reason for the decline in pH in the CPN sample during the first 20 days (Gao et al., 2010). Many spoilage organisms and pathogens were inhibited and assisted in reducing the nitrite accumulation in the fermentation environment at low pH (Wang et al., 2018b). These results suggested that *L. sakei* inoculation could be conducive to improving microbiological quality and safety of chili sauce.

3.2 Effect of the starter culture on nitrite depletion in chili sauce

Effects of *L. sakei* inoculation on nitrite, putrescine and histamine concentration in chili sauce are shown in Figure 2. The nitrite concentration in CPS and CPN samples was 7.84 mg/kg and 5.42 mg/kg on the 40th day, respectively. The nitrite concentration in the CPN sample was significantly lower than that of the CPS sample as shown in Figure 2 ($p < 0.01$). The nitrite in chili sauce is mainly derived from nitrate in chili which is reduced to nitrite by nitrate-reducing bacteria with nitrite reductase activity during fermentation. It is widely known that nitrite could oxidize Fe^{2+} in hemoglobin to Fe^{3+} state, resulting in a nitrite poisoning. Furthermore, nitrite might react with amines to form nitrosamines which could bring about a positive correlation between an increasing risk of gastric, esophageal, and bladder cancers (Wang et al., 2015; Yan et al., 2008; Zhang et al., 2018). Thus, excessive intake of nitrite could induce the risk of nitrite poisoning and cancer. *L. sakei* inoculation could inhibit the growth of nitrite-reducing bacteria, resulting in the reduction of nitrite formation in chili sauce.

3.3 Effect of the starter culture on biogenic amine formation in chili sauce

The histamine concentration in the chili sauce fermented by *L. sakei* was significantly ($p < 0.01$) lower than that of spontaneous fermentation as shown in Figure 3. Putrescine and histamine concentration in the CPN sample was 3.85 and 2.12 mg/kg on the 40th day, respectively. In contrast, putrescine and histamine concentration in the CPS sample was 6.25 and 4.23 mg/kg on the 40th day, respectively. Biogenic amines in chili sauce are mainly attributed to the presence of decarboxylase-producing bacteria with decarboxylase activity, which may be introduced by contamination during fermentation. It has been reported that a high intake of dietary biogenic amines may increase the risk of cancers, in which histamine and putrescine are highly toxic biogenic amines (Wang et al. 2018a). The consumption of histamine at a dose exceeding 100 mg/kg could cause symptoms of acute poisoning (Shalaby, 1996). The high level of putrescine has been associated with a risk of developing colorectal adenocarcinomas. It has been reported that *L. sakei* could produce histamine-degrading enzymes (Lu et al., 2015) and inhibit the growth of decarboxylase-producing bacteria, resulting in lowering of histamine and putrescine levels to less than 5 mg/kg and 5 mg/kg in fermented foods, respectively (Bover-Cid et al., 2001; Wang et al., 2021). These results indicated

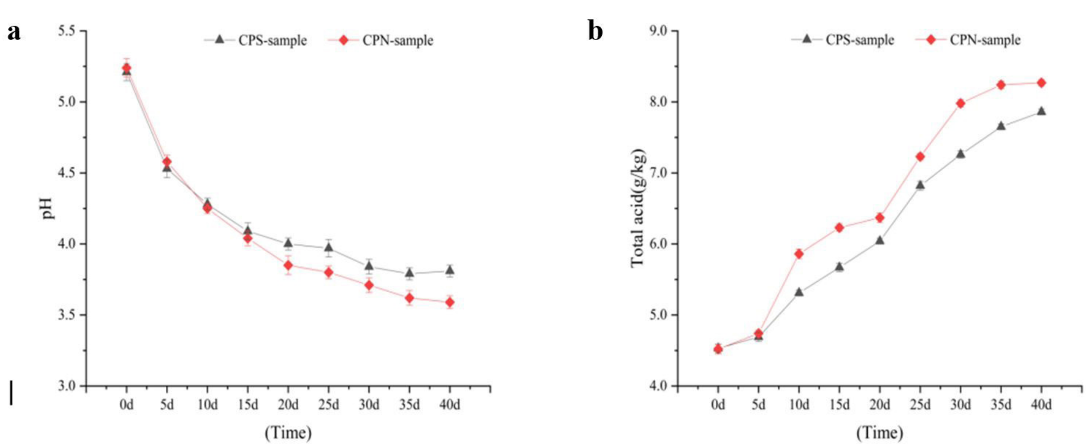

Figure 1. Effect of starter cultures on the pH (a) and total acid levels (b) in fermented chili sauce during fermentation.

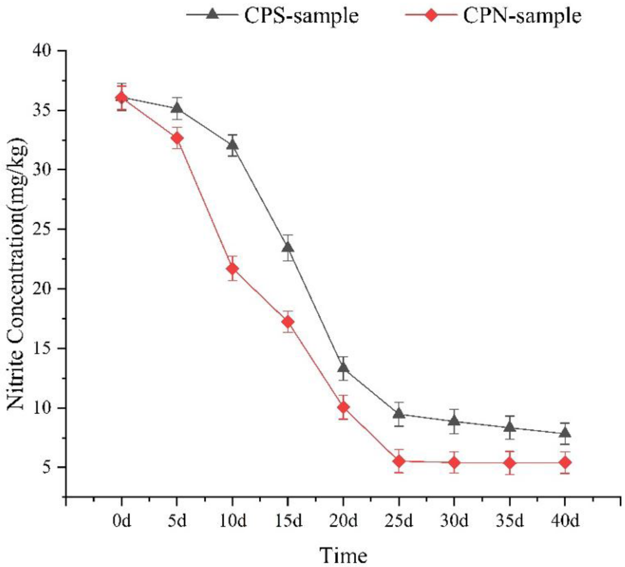

Figure 2. Effect of starter cultures on the nitrite levels in fermented chili sauce during fermentation.

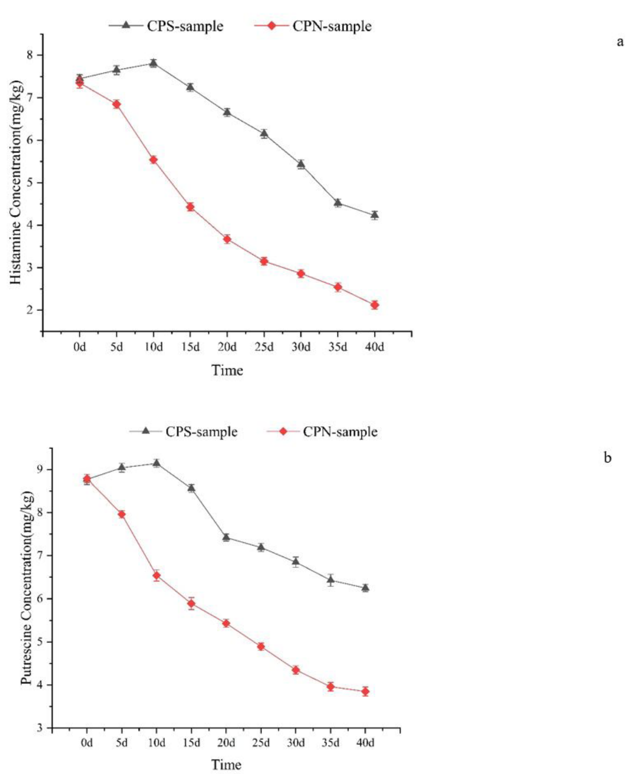

Figure 3. Effect of starter cultures on the histamine (a) and putrescine levels (b) in fermented chili sauce during fermentation.

that *L. sakei* inoculation could improve the safety parameters of chili sauce by decreasing the accumulation of nitrite, putrescine and histamine.

3.4 Effect of the starter culture on sensory evaluation

After fermentation, sensory evaluation of the CPS and CPN samples was performed based on the average scores for color,

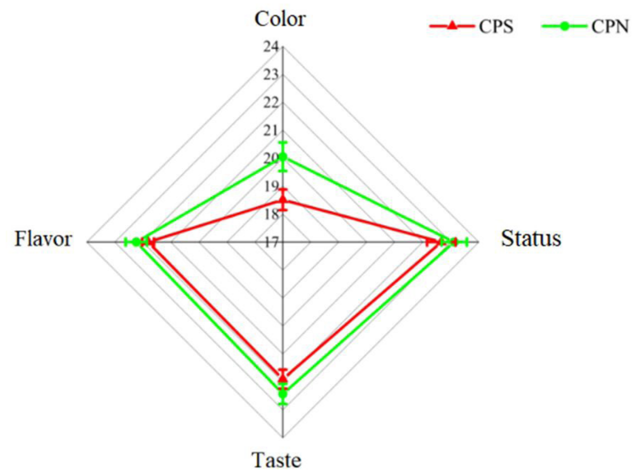

Figure 4. Sensory evaluation of CPS and CPN samples based on the average scores for color, flavor, status and taste. CPS: control sample during fermentation; CPN: sample inoculated with starter culture during fermentation.

flavor, status, and taste (Figure 4). These results indicated that the CPN samples showed improvement in color, flavor, status, and taste of chill sauce compared with the control. Unexpectedly, the color scores of the chili sauce for the CPS and CPN samples were 18.51 and 20.05, respectively. It was speculated that the color of the chili sauce was related to the pH value. The higher the pH was, the darker the color of the chili sauce was. *L. sakei* could produce organic acids and decrease the pH, thereby improving the color of the chili sauce, resulting in a more intensely red-colored sauce. These results showed that the chill sauce inoculated with *L. sakei* had a wide sensory appeal among consumers.

4 Conclusions

Chili sauce is a representative and traditional fermented food in China. *L. sakei* was used as a starter culture for fermentation because it improves the safety profile of fermented foods by producing organic acids and bacteriocin that acts as natural preservative. These results revealed that the levels of harmful nitrite and biogenic amine in the CPN samples were significantly ($p < 0.01$) lower than that of the control. Collectively, these results revealed that *L. sakei* inoculation in the chili sauce during fermentation was conducive to improving the safety profile of the product.

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Supplementary Material

Supplementary material accompanies this paper.

Supplemental Table 1 Sensory score sheet of chili sauce.

This material is available as part of the online article from https://www.scielo.br/j/cta