

Effects of Lactic Acid Bacteria Fermentation on the Quality of Little Yellow Croaker

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Abstract: This research was to study the effects of lactic acid bacteria fermentation on the quality of little yellow croaker. The effects of the LAB starter composed of *Lactobacillus plantarum* and *Lactobacillus acidophilus* on the quality of little yellow croaker were studied through a 72 h fermentation process in this study. During 72 h fermentation at 30°C, little yellow croaker inoculated with the LAB starter not only resulted in a rapid pH decrease and suppression of spoilage bacteria, but also receded chemical changes such as total volatile base nitrogen and biogenic amines, its texture profile and whiteness also satisfying. Besides, Scanning Electron Micrograph (SEM) images indicated some microstructure changes in LAB fermentation. The results demonstrated that the LAB starter could be developed as bio-preservatives to improve the quality of little yellow croaker in storage.

Keywords: Bio-preservation, lactic acid bacteria, little yellow croaker

INTRODUCTION

Little yellow croaker is an important fish species of seafood in China, which is rich in protein, fat, calcium, phosphorus, iron, vitamin A and other nutrients. Apart from that, it is easy to get corrupted in ordinary condition (temperature, water activity, humidity), which is related to chemical and microbiological mechanisms (Gennari *et al.*, 1999). Dalgaard (1995) put forward the concept of Specific Spoilage Organism (SSO) which leads to specific corruption. Spoilage bacteria is the main cause of off-odours and off-flavours and most of the volatile compounds are produced by bacteria metabolism, including TMA (value of trimethylamine), volatile sulphur compounds, aldehydes, ketones, esters, hypoxanthine as well as other low molecular weight compounds (Pilar *et al.*, 2008).

Lactic Acid Bacteria (LABs), which is generally accepted as safety, produce metabolites, such as lactic acid, hydrogen peroxide, diacetyl, acetoin and other organic acids, acting as bio-preservatives by altering the intrinsic properties of the food to such an extent as to actually inhibit spoilage microorganisms (Reis *et al.*, 2012). In dry fermented sausage with additional curing salt, the Gram-negative bacteria are inhibited while the LABs and micrococccaceae are boosted and at the end of storage, LABs became predominant (Truelstrup *et al.*, 1996; Leroi *et al.*, 2001; Lyhs *et al.*, 2007; Jaffrès *et al.*, 2008). There are few reports have been on LAB

used for sea-fish fermentation, while most related references were about sea-fish sauce or ready-to-eat sea-food.

So far, the storage of marine fish is mainly based on low temperature and freezing preservation which is of high cost. LABs have a major potential for use in bio-preservation because they are generally regarded as safety and during storage they could naturally dominate the microflora in many foods (Stiles, 1996). The main purpose of this research was to determine the effects of LAB on the little yellow croaker, studying the fermentation process, providing information for the further bio-preservation by LAB.

MATERIALS AND METHODS

The little yellow croaker was purchased from Wenzhou Village in Hangzhou, China. *Lactobacillus plantarum* ZJU10012 and *Lactobacillus acidophilus* ZJU10023 were stored in our laboratory. All five standard biogenic amines were purchased from Sigma, China; all the other chemicals are of analytical grade.

Before the experiments, two bottles with 1000 mL brine of 5% salt (w/v) and 5% sucrose (w/v) were made by strict sterilization in 121°C for 20 min. The activated strains were centrifuged at 3000 g for 20 min in high speed refrigerated centrifuge; the precipitation was taken and washed with sterilization water, flushed 2-3 times and diluted into 10⁷-10⁸ cfu/mL.

Two experimental groups were set. 20-30 pieces of little yellow croaker were placed into each one of the two bottles above. The first bottle was inoculated with 3% (v/v) *Lactobacillus acidophilus* ZJU10012 and 3% (v/v) *Lactobacillus plantarum* ZJU10023 together. The second was untreated as control group.

The samples were then fermented at 32°C and relative humidity 80% for 72 h, taken every 24 h for analysis. The control sample has been stopped after 24 h for its serious corruption.

Total viable counts were determined according to the method of Song *et al.* (2012). LAB counts were determined in Man Rogosa Sharpe (MRS) media. Enterobacteriaceae amounts were determined in Violet Red Bile Glucose agar (VRBG) media in 37°C for 24 h. Results were expressed as log colony forming units per gram sample (log CFU/g sample).

Fish flesh (10 g) was dispersed in the 100 mL of distilled water and stirred for 30 min and then the mixture was filtered. The pH of the filtrate was measured using a pH metre.

The extraction of BAs in fish fillets was according to Hu *et al.* (2007a, b) with appropriate modification (Garcia-Garcia *et al.*, 2000). BAs were extracted from little yellow croaker with 5% (w/v) trichloroacetic acid twice. Pre-column amine derivatisation was done with dansyl-chloride (De Mey *et al.*, 2012). Ten μ L volume was injected into HPLC (LC-2010A SHIMADZU) each time for analyzing.

The HPLC column was phenomenex C₁₈ (250×4.6 mm, Synergi 4u Hydro-RP 80A). The HPLC operating condition was: column temperature was 30°C, flow rate was 1 mL/min and sample volume injected was 10 μ L, 254 nm UV detector.

Standard BAs, namely, histamine, putrescine, tyramidine, spermidine, spermine were purchased from Sigma-Aldrich.

TVB-N was measured using microdiffusion methodology. TVB-N value was determined according to the consumption of hydrochloric acid. It referred to Conway micro-diffusion technique (Cobb and Thompson, 1973).

TPA measurements were carried out with TA-XT2i Stable Micro Systems Texture analyzer. Fermentation fillet was cut into 2 cm thick and detected the fillet of hardness, springiness, adhesiveness, chewiness and resilience with probe P50. TPA was measured following the testing conditions: measurement speed is 2 mm/s, test rate is 10 mm/s, after-test rate is 10 mm/s, strain 40%, time interval between first and second stroke 5 sec.

The reflected color of samples was measured using a KONICA MINOLTA CR-400 Japan (WSC-S: a tristimulus color analyzer). In this system, the degree of lightness, redness, greenness, yellowness, or blueness are represented by L^* , $+a^*$, $-a^*$, $+b^*$, $-b^*$, respectively and are reported as L^* , a^* and b^* values.

The instrument was calibrated using a standard white tile ($Y = 87.0$, $x = 0.3165$, $y = 0.3235$) placed under the orifice of the instrument. The color of each treatment was recorded at the same spots of samples. The efficacy of each treatment to influence the color of little yellow croaker was determined by calculating whiteness, thus:

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + (a^*)^2 + (b^*)^2]^{1/2}$$

Each specimen was formed or cut into a conical shape (approximately 2×2×2 mm, length×width×height) with a Stanley knife.

Microstructure was illustrated by SEM. The fish fillets were soaked in 2.5% glutaraldehyde (v/v), then washed in 0.1 M phosphate buffer, pH 7.2, dehydrated in increasing concentrations of acetone (30, 50, 70, 90, 95, 100%, respectively), critical-point-dried, sputter-coated with gold/palladium in a metallizer and scanned by SEM (Jeol, JSC 6400, Akishima, Tokyo, Japan) at 20 kV. A large number of micrographs were taken in order to select the most representative ones.

All statistical analyses were performed using SPSS statistic program (version 19.0 for windows, SPSS 2012). Comparison of values was carried out using Analysis of Variance (ANONA).

RESULTS AND DISCUSSION

During the 72 h fermentation, the pH of little yellow croaker decreased from 7.33 to 4.12. The control sample has reached to 8.25 after 24 h, accompanied with rapid deterioration and had to stop fermentation because of its serious corruption in 24 h. LABs could produce many antimicrobial materials such as organic acids (mainly lactic and acetic acids), hydrogen peroxide and bacteriocins thus giving the fermentation brine acidic condition (Asiedu and Sanni, 2002; Yin *et al.*, 2005).

Spoilage organisms, especially enterobacteriaceae and pseudomonas, belonging to amino-positive microorganisms, use free amino acids and nitrogenous compounds to produce TVB-N (Liu *et al.*, 2010). TVB-N is the most useful and important index for identifying the spoilage in fresh and fermented fish products. Fish is fresh if the TVB-N is in range of 15-25 mg/100 g. For the experimental sample, deterioration has not been observed during 72 h fermentation process and the TVB-N increased from 10.976 to 22.696 mg/100 g. However, the control sample has gotten extremely poor quality and unbearable smell, its TVB-N hitting to 76.13 mg/100 g in 24 h.

The results of the present study showed that the low pH value and better growth of LAB leads to the lower value of TVB-N (Fig. 1), coinciding with microbial analysis in 3.3 that LAB fermentation suppress the growth of spoilage bacteria.

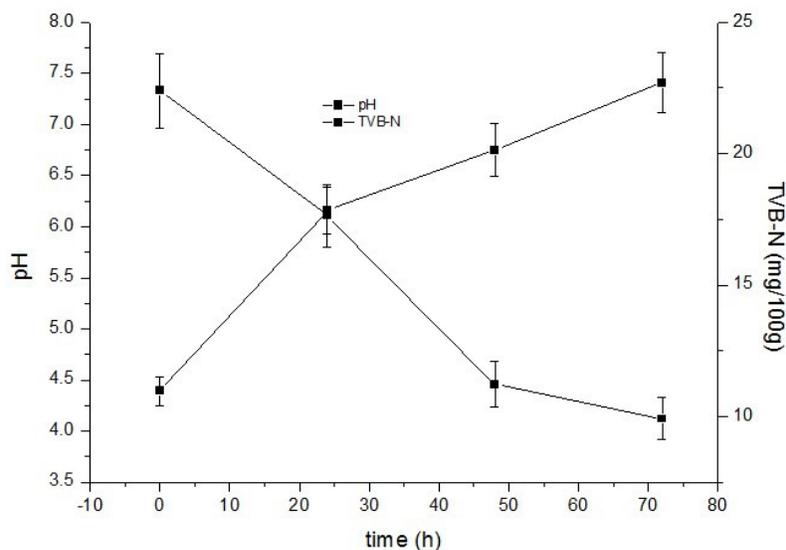


Fig. 1: Results of TVB-N and pH of experimental sample in 72 h

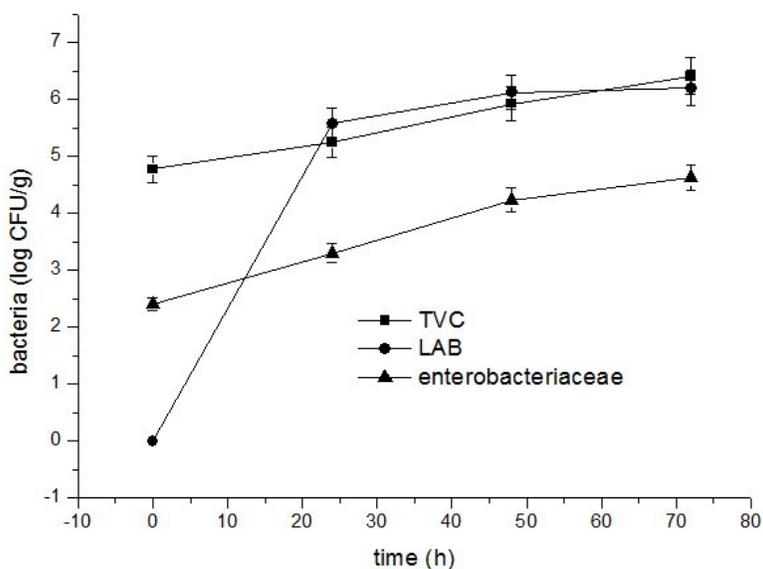


Fig. 2: Results of microorganism of experimental sample in 72 h

Five BAs are measured in this experiment and results are showed in Table 1. Bas were very low in fresh fish fillets or raw material (Pons-Sanchez-Cascado *et al.*, 2005). During 24 h spontaneous fermentation, a dramatic rise occurred in control sample; the content of each BA was far more than upper standard line (Table 2). BAs were a main contributor of TVB-N (Liu *et al.*, 2010). Many Enterobacteriaceae and Pseudomonas possess histamine, lysine and ornithine decarboxylase activities, which lead to considerable histamine, cadaverine and putrescine produced (Durlu-Ozkaya *et al.*, 2001; Liu *et al.*, 2010). Histamine as the most important BA in the experimental sample was still below security target 50

mg/kg (Liu *et al.*, 2010) after 72 h fermentation. Putrescine and tyramine showed similar tendency to histamine in the experimental sample. Low pH values suppress the amine-positive microorganisms, especially enterbacteriaceae and consequently reduce BAs formation in experimental sample, thus TVB-N formation inhibited (Liu *et al.*, 2010), which is consistent with microbiological analysis results below.

The changes in the microbial flora are shown in Fig. 2. The total microbial amount of control sample was incalculable after 24 h which had significant difference with that of the experimental sample.

LAB amount of the experimental sample got rapid increase in the 0-24 h period, hitting to 5.61 ± 0.16 log

Table 1: Results of BA values of experimental sample in 0 and 72 h

Time (h)	Biogenic amines (mg/kg)				
	Putrescine	Histamine	Tyramine	Spermidine	Spermine
0	12.15±1.07	31.87±2.32	29.65±2.17	1.09±0.11	1.21±0.09
72	37.81±3.02	49.32±4.21	38.12±2.73	1.22±0.08	1.83±0.10

Table 2: Results of BA values of control sample in 24 h

Time (h)	Biogenic amines (mg/kg)				
	Putrescine	Histamine	Tyramine	Spermidine	Spermine
24 h	93.72±8.72	187.39±20.91	78.62±6.38	5.81±0.65	6.08±0.79

The control sample experiment has been stopped after 24 h for extremely corruption

Table 3: Results of TPA and whiteness of experimental sample during 72 h

Color and TPA	Samples			
	0 h	24 h	48 h	72 h
L	46.720±3.290	56.220±4.760	63.070±5.380	65.430±4.930
a	1.670±0.090	2.320±1.720	3.450±0.270	3.610±0.310
b	1.740±0.080	5.790±0.430	8.870±0.720	8.930±0.610
Whiteness	46.665±3.290	55.778±4.360	61.834±5.890	65.113±5.770
Hardness (g)	163.154±9.630	533.415±40.590	651.870±53.100	865.017±72.180
Springiness (mm)	0.365±0.021	0.584±0.034	0.841±0.065	0.989±0.067
Cohesiveness	0.856±0.034	0.734±0.046	0.604±0.051	0.362±0.022
Gumminess (g)	134.722±9.850	205.396±17.310	465.808±32.150	641.098±43.330
Chewiness (g.x.mm)	201.065±18.900	361.649±25.430	558.376±40.750	652.055±51.190

a*: Redness; L*: Lightness; +b*: Yellow trend; -b*: Blue trend

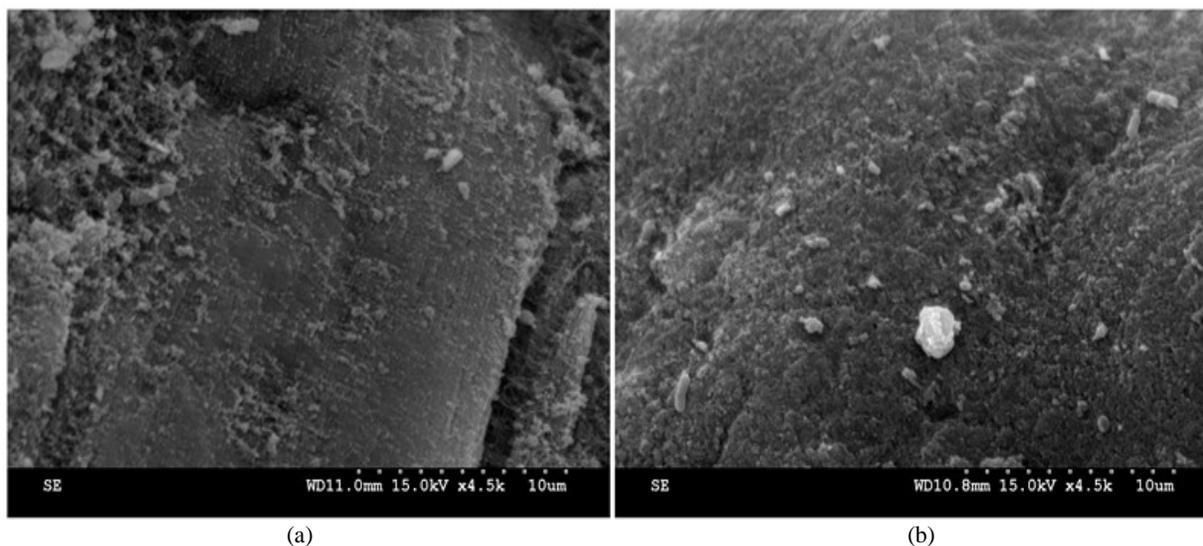


Fig. 3: Scanning electron micrograph (SEM, ×4500) of, (a) little croaker fillets no inoculated with LAB in 0 h, (b) little croaker fillets after 72 h fermentation of LAB

cfu/g at 24 h and keeping the level of 5.0-6.5 log cfu/g during 24-72 h period. It implies that LAB inoculated in the fish fillets can adapt to the real conditions and became the dominant microorganism during the fermentation process (Mokhtar *et al.*, 2012).

LAB took over the overwhelming superiority thus leading to the low amount of spoilage bacteria enterobacteria. In this research, enterobacteria has been the Specific Spoilage Organism (SSO) during corruption process, thus its amount in control sample was incalculable after 24 h, while in experimental

sample the LABs controlled the enterobacteria at level of 4-5 log cfu/g. Low pH has certain effects of inhibiting enterobacteria, which was in agreement with the results. The antagonistic effects of LAB were mainly due to its ability of lowering pH value in food, competition for nutrients and the production of inhibitory metabolites, thus controlled the spoilage bacteria (Stiles, 1996).

Table 3 has indicated that in the 72 h fermentation process, the values of L*, a* and b* of the experimental sample distinctly get increased, thus the formula results

of whiteness rose gradually, indicating fish fillets degraded by the LABs. Being whiteness could be regarded as the color quality of little yellow croaker fillets improved. The experimental sample after 72 h fermentation also show good flavor, while the control sample has presented unbearable appearance after 24 h spontaneous fermentation with heavy fishy smell and awkward gray color.

Compared to initial fish meat, the TPA results indicate that experiment sample become well in the index of hardness, springiness, adhesiveness, cohesiveness, chewiness and resilience than initial fish meat. What is more, fermented samples showed better than raw material in some characteristics, the hardness value got sharply decrease and chewiness value got apparently increase.

Scanning Electron Microcopy (SEM, $\times 4500$) was carried out to analyze the inner microstructure of little yellow croaker fillets.

The surface morphological characteristics of little yellow croaker fillets in 0 and 72 h have been characterized by SEM. The SEM images below showed muscle profile in 72 h became more loosen than that in 0 h. Some thalli particles of LAB adhered in the surface of muscle, getting compactness smaller.

Further study is required to determine how the analysis of SEM could be used to qualitatively explain the effect of LAB fermentation on microstructure and the association between microstructure and marine fish corruption extent (Fig. 3).

CONCLUSION

In this study, the relation between the growth of LABs and the inhibition of fish spoilage is well in agreement with the results of each index. The control sample without inoculated with LAB showed undesirable results that pH value failed to rapid decline, thus unbearable corruption of little croaker fillets stopped its experiment. In experimental samples inoculated with LABs, spoilage bacteria were obviously inhibited and its TPA and whiteness were in favorable level.

In conclusion, the results showed that LABs could be used as bio-preservative. Little croaker fillets is one of the important marine fishery resource, the present research lay foundation for further study of applying LABs in marine fish preservation and process technology.

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