

## Effect of Inoculating Lactic Acid Bacteria Starter in Low-Salt Pickle Process of Zhacai

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**Abstract:** The traditional pickle process of Zhacai was thought unsafely and unstable, because of the high salt and nitrite contents. The study has shown a method to solve these problems. One Lactic Acid Bacteria (LAB) strain, *Lactobacillus plantarum* was used as starter in the low salt pickle process of Chinese Zhacai. The LAB inoculation amounts were 2, 5, 8%, respectively and the group without LAB inoculation was set as the control. The inoculation groups were added with 8% salt, while the control group 10%. The dynamic changes of physical and chemical parameters of two groups were detected during the pickle process. The results showed that: pH value of the inoculated groups declined rapidly, the lowest pH value was 3.61; the LAB became the predominant bacteria strains during the pickle and the colony amounts of the inoculated groups could be up to  $6.32 \log_{10}^{cfu}/mL$ ; the nitrite content of each group kept rising and the nitrite peaks of the inoculated groups appeared 3 days in advance, but the peak values were nearly half of the control group; amino nitrogen contents were lower but finally higher than control group and the highest value could reach 0.46 g/100 mL finally. Therefore, the inoculated groups with the lower salt could be more safety and better flavor. This study provided important reference to optimize the pickle fermentation process of Chinese Zhacai.

**Keywords:** Inoculum pickle, Lactic Acid Bacteria (LAB), probiotic, tuber mustard, zhacai

### INTRODUCTION

Zhacai belonging to tuber mustard is one famous kind of Chinese traditional pickle vegetable for more than two thousand years. Zhacai is planted widely in China and it has been the popular dish because of the good taste and flavor. Adding numbers of salt is used as the traditional Chinese method to produce Zhacai, which is in order to inhibit the growth of spoilage microorganisms, but this way can bring many disadvantages, such as high salinity against physical health and a large number of nitrite appearing in the process. Nitrite combined with amine may generate carcinogens, nitrosamines (Drabik-Markiewicz *et al.*, 2012).

The Lactic Acid Bacteria (LAB) is known as a kind of probiotic (Gajewska and Blaszczyk, 2012). LAB has been widely recognized the characteristics of antibacterial, reducing the content of nitrite and biological amines and producing superior fermentation flavor. LAB combined with low salt has been used for vegetables production in some vegetable fermentation industry successfully. LAB can obviously limit the formation of nitrite during Paocai fermentation (Yan *et al.*, 2008). LAB can also constrain buildup of

dangerous biogenic amines in fermented vegetables during storage (Rabie *et al.*, 2011).

In the present study, we monitored the changes of LAB, pH, nitrite concentration and amino nitrogen during Zhacai pickle process under spontaneous and LAB starter inoculated conditions. From the results, we attempted to establish a new process for pickle Zhacai.

### MATERIALS AND METHODS

**LAB strain, lactobacillus:** *Plantarum* was provided by Gaofuji Biological Technology Company in Si Chuan province of China.

The raw materials for the fermentation trials were supplied by Tongqianqiao Food Company in Ningbo of China, including Zhacai and the common salt.

This study was conducted at Tongqianqiao Food Company in Ningbo of China from March 20<sup>th</sup> to June 20<sup>th</sup>, 2012.

#### Methods:

**Zhacai pickle process:** Zhacai was washed and put into the pickle-pool, 300 kg each pool. For the inoculation groups, 5% salt was added in the first pickle process and 3% salt in the second pickle process. 2, 5 and 8%, respectively. LAB was inoculated into Zhacai

that had been divided into four groups. The control group was added with 5% salt for both-pickle. The samples of brine were detected every 2 days.

**Determination of pH:** Decrease in pH of the brine was measured with a pH meter (PHS-9V, Huaguang Company of Hang Zhou).

**Determination of nitrite concentration of zhacai:** Nitrite content of Zhacai was determined using colorimetric nitrite assay as described by Akemi and Kuniyasu (1979). Approximate 3 g Zhacai was crushed, deproteinated and defatted by precipitation with 10 mL ZnSO<sub>4</sub> (0.42 mol/L) followed by filtration. One mL each of the three color development reagents, namely 0.2% sulfanilamide, 0.1% N-1-naphtyethylene diamine dihydrochloride and 44.5% HCl was added sequentially to the filtrates. The mixtures were then kept at room temperature for 5 min. OD of the colored mixtures was read at 538 nm against the reagent blank. To construct a standard curve, 0-3000 µg/mL sodium nitrite solutions were subjected to the similar color development and OD measurement steps.

**Determination of Amino nitrogen concentration of Zhacai:** Twenty mL diluted samples (20-fold dilution with distilled water, w/v) were mixed with 60 mL H<sub>2</sub>O and titrated to pH 9.6 with NaOH (0.05 mol/L) before 10 mL formalin solution (37%) was added. The consumed volume was recorded to determine total titratable acids of samples. The samples were finally titrated to pH 9.2 with NaOH (0.05 mol/L) (Xianli *et al.*, 2011).

**Determination of LAB counts.** A 10 g sample was blended in 90 mL sterile NaCl solution (0.85%, w/v) in a 500 mL Erlenmeyer flask using a lab-blender for 2 min at “normal” speed and then a 1:10 dilution was made. Sequential decimal dilutions of the homogenate were made successively afterward. (0.1 mL) of the appropriate decimal dilutions were plated onto the MRS agar and incubated at 37°C in a candle jar for 48-72 h. The colonies appeared on the selected plates (50-300 colonies per plate) were counted as Colony Forming Units (CFU) per mL of the sample (Tamang and Sarkar, 1996).

## RESULTS AND DISCUSSION

**LAB counts during the process:** LAB of inoculation groups grew more quickly than the control group (Fig. 1), the number of LAB could exceed 8.01 log<sub>10</sub> cfu/mL at the end of first stage on 12 days. After all the groups were added salt again, LAB of inoculation groups declined slightly, gradually kept stability. When the pickle processes were over, the maximum number of LAB of inoculation groups could be up to 6.32

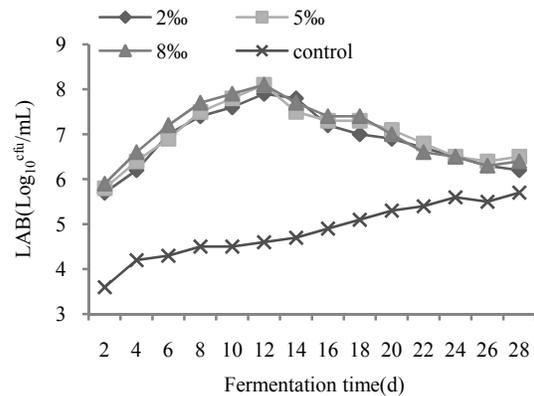


Fig. 1: The dynamic change of LAB

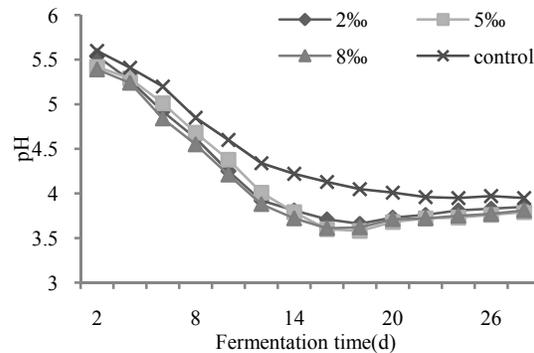


Fig. 2: The dynamic change of pH

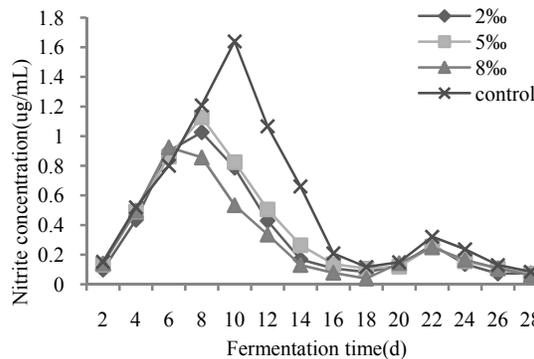


Fig. 3: The dynamic change of nitrite

log<sub>10</sub> cfu/mL. While LAB of the control group increased slowly, finally it could be only 5.53 log<sub>10</sub> cfu/mL.

**The pH value change trends:** Change trends of pH values were shown in Fig. 2. When the pickle started, pH of each group declined gradually. But since the beginning of the pickle process, the pH values of inoculation groups were obviously lower than the control group. Especially at the end of first stage, the inoculations could reach 3.53, while the control could only be 4.27. At the end of Zhacai pickle process, the

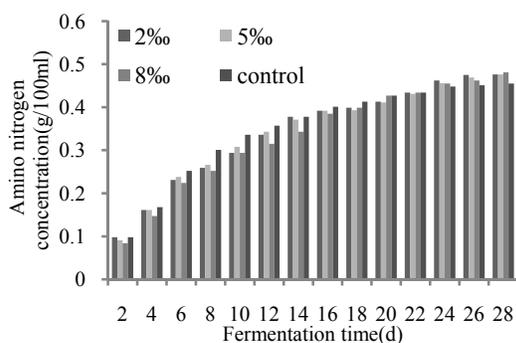


Fig. 4: The dynamic change of amino nitrogen

pH values of inoculation groups were approximately 3.61 while that of control group was approximately 3.95.

**Nitrite change trends:** The trend of nitrite was showed that there were nitrite peaks for every group (Fig. 3). The nitrite content of each group kept rising at the start, but nitrite concentrations of inoculation groups were significantly lower than that of control group during the whole pickle process. In the first pickle stage, nitrite peaks of the inoculation groups appeared on 8 day, while the control appeared on 10 day. The inoculation groups reached the peak ahead of 2-3 days compared to the control group and the peaks were only half of the control group. In the second stage, each group appeared nitrite peak almost at the same time, but inoculation groups were lower than the control one.

**Amino nitrogen change trends:** Amino nitrogen content kept rising gradually in the progress (Fig. 4). The inoculation groups were obviously less than the control group during the pickle process and the group with the maximum inoculation had the least content of amino nitrogen. But at the end stage of the process, amino nitrogen content of inoculation groups increased faster, finally the highest content of amino nitrogen could reach 0.46 g/100 mL.

## CONCLUSION

The inoculation groups with low-salt could make LAB become the dominant strains in the early time of the pickle process, so that the probiotic grew rapidly and soon acidified, with the lowest pH 3.61. On one hand, LAB inhibited pathogen growth. On the other hand, the rapidly falling acidity could activate the nitrite enzyme (Oh *et al.*, 2004), which resulted that the peak of nitrite appeared in advance compared with the control and with little contents of amino nitrogen, thereby reduced the formation of nitrosamines significantly. What's more, LAB could help to cut down nitrite peak obviously in the pickle process and the peak values were nearly half of the control group. In the end, the number of LAB and amino nitrogen content were both higher than the control group. The maximum

number of LAB could be up to  $6.32 \log_{10}^{cfu} / \text{mL}$  and the most content of amino nitrogen could reach 0.46 g/100 mL. All these contributed to the flavor formation of Zhacai (Klacanova *et al.*, 2010).

Therefore, the inoculation pickle process with low-salt was obviously better than the high-salt pickle process. However, little differences were found between inoculation groups, the maximum inoculum concentration group was slightly better than the other two groups. Inoculation amount needed to optimize in further experiment.

## ACKNOWLEDGMENT

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