

Biochemical Changes in the Fermentation of the Soy Sauce Prepared with Bittern

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Abstract: A new bittern soy sauce was developed; the overall aim of this study was to characterize the quality of the soy sauce. Biochemical changes occurring during experimental soy sauce fermentations were described. Results revealed that, the contents of amino nitrogen, reducing sugars and enzyme activity were slightly lower in soy sauce prepared with bittern than with traditional one. No marked difference in pH was noted between the two types of soy sauce.

Keywords: Bittern, fermentation, soy sauce

INTRODUCTION

Soy sauce is a traditional fermented condiment commonly consumed in China, Korea and Japan. And it is a popular condiment all over the world in recent years (Lioe *et al.*, 2010). Studies have shown that soy sauce has a promotive effect on human health, such as on iron absorption in human subjects (Baynes *et al.*, 1990).

The fermentation of the soy sauce is very complicated, including starch saccharification, sugar degradation, alcoholic fermentation, proteolysis, aroma formation, pantothenic acid reaction and Maillard reaction etc. The conventional batch process of brewing soy sauce starts with a solid-state fermentation of *Aspergillus* species on a mixture of soybeans and wheat with a certain ratio (Van der Sluis *et al.*, 2001). During an aerobic fermentation for 2-3 days at 30°C, *Aspergillus* produces extracellular enzymes. After that, the moulded raw materials are mixed with saline water. The brine solution is kept around 40-50°C. In the brine solution the *Aspergillus* enzymes continue to hydrolyze the soybeans and wheat and as a result, the soy sauce was obtained (Yong and Wood, 1977).

In this study, salt is placed by the wasted bittern in the fermentation, which is environment-friendly. Three different ratios of salt were placed by the bittern, 50, 75 and 100%, respectively. We determined the biochemical changes every 4 days.

MATERIALS AND METHODS

The fermentation of the bittern soy sauce: The raw materials were soybeans, wheat and bran at the ratio of 3:1:1. All of the soy sauce was fermented in the same incubator for 21 days in the library of Zhejiang University; the temperature was gradually increased to 50°C then reduced slowly, between 40 and 50°C.

The bittern used in this experiment was produced by the Jingyangguan Pickles Factory in Xiaoshan, China. All the other chemicals are of analytical grade. All of these experiments were conducted in the library of Zhejiang University.

Methods:

Determination of pH: Ten mL of deionizer water was added into 10 g of soy sauce mash, pH was measured by the acidity meter after being well stirred.

Determination of amino nitrogen and total acidity: To determine total acidity as lactic acid, 60 mL of distilled water was added to 5 mL of sample and titrated with 0.05 mol/L NaOH to pH 8.2. For amino nitrogen, 10ml formaldehyde was added and kept titrating until the pH reached 9.2 on the basis of the total acidity.

Determination of reducing sugar: The estimation of the reducing water was carried out by Nitro Salicylic acid (DNS) reagent (Sengupta *et al.*, 2000). DNS reagent, which contained (w/v) 1% DNS, 2% NaOH and 20% sodium potassium tartrate, was prepared by dissolving each component in the minimum volume of water and then mixing DNS and tart rate together. Alkali solution was added slowly to the mixture and the final volume was adjusted with water. In the estimation, 1ml of sample mixed with 1.5 mL DNS reagent were kept in a boiling water bath for 10 min. Color intensity was measured at 540 nm.

Determination of amylase: One mL of diluted sample and 1ml of citric acid buffer (pH5.6) were mixed in the tube. After heating at 40°C for 10 min, 1 mL of starch (1%) was added into all of the tube, then after the

insulation for 5 min, 1.5 mL, the reducing sugar was measured. Black ones were added into 1.5 mL DNS before the first heating.

Determination of protease: Protease activity was carried out by the method of Folin-phenol. One mL of 1% (w/v) casein in sodium Phosphate Buffer (PBS, 0.02 M) and 1 mL sample were pre-incubated at 40°C, respectively. After 2 min, the casein was incubated with the sample at 40°C for 10 min and then unhydrolyzed protein was precipitated with 2 mL of 0.4 mol/L TCA and softly shaken. After centrifugation at 10,000 rpm for 10 min, 4 mL Na₂CO₃ was added to 1 mL supernatant followed by 1 mL Folin-phenol reagent and then immediately shaken up. The reaction mixture was allowed to stand for 20 min at 40°C before measuring the absorbance at 640 nm by a spectrophotometer against blank samples (Li *et al.*, 2005).

RESULTS AND DISCUSSION

Changes of pH and total acidity: The pH of all the soy sauce decreased rapidly during the first 17 days, while increased slightly in the last four days (Fig. 1). The pH of soy sauce with the bitter was smaller than the traditional one for the highly acid of the bitter and the more the bitter, the smaller the pH would be the gap between the bitter and the traditional one became smaller in the last days. At the start of fermentation, microorganism such as lactic acid bacteria decomposed carbohydrates and fats to produce small molecule organic acids such as lactic acid, acetic, decomposed the protein to produce free amino acid. As a result, pH reduced. While After a period of time, microbial cells began to autolyze when the fermentation environment became not suitable for the growth of the microorganism, accumulation of free fatty acid, amino acids and peptides. Alcohols produced by the yeast will combined with the acid to form esters, the metabolism of lactic acid bacteria became slow or stopped. The change of the total acidity was just the opposite with the pH (Fig. 2).

Changes of reducing sugar: Figure 3 shows the changes in reducing sugar content during the fermentation. The contents of reducing sugar increased rapidly as the fermentation began, reaching their maximum after 5 days (Chou and Ling, 1998). The rapid increase may be attributed to the action of fungal amylase activity, amylase and glucoamylase hydrolyzed of starch in raw materials to produce reducing sugars. However, as the fermentation proceeded, amylase activity was decreased due the fast use and consumption by the microorganisms. On the one hand,

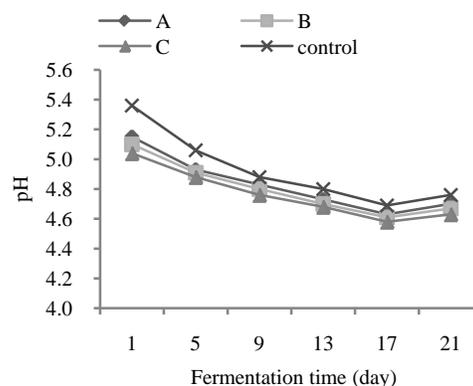


Fig. 1: The pH of soy sauce in the fermentation
A: Half of the salt being placed by the mustard tuber bitter; B: 75% of the salt being placed by the mustard tuber bitter; C: All of the salt being placed by the mustard tuber bitter; Control: The soy sauce prepared with salt

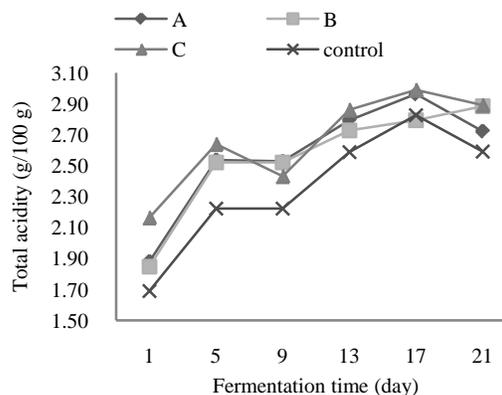


Fig. 2: The total acidity of soy sauce in the fermentation

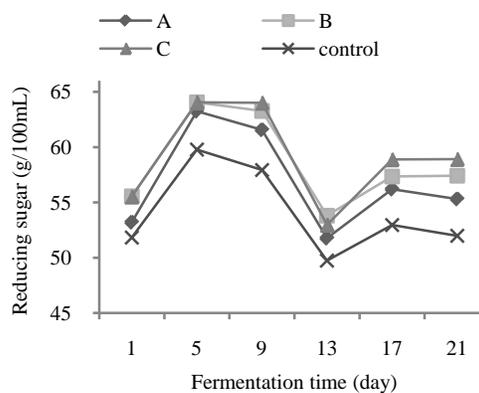


Fig. 3: The reducing sugar of soy sauce in the fermentation

reducing sugars provided a carbon source for microbial growth; on the other hand, Maillard reaction consumed reducing sugar (Chou *et al.*, 1988b). It was noted that

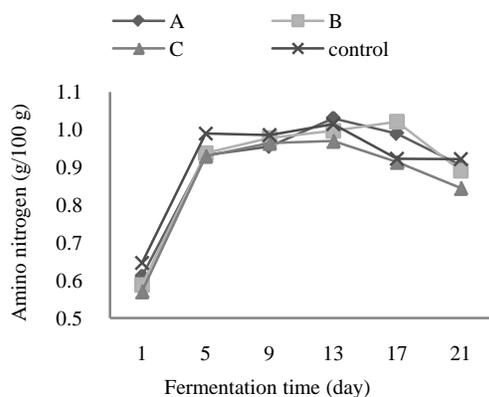


Fig. 4: The amino nitrogen of soy sauce in the fermentation

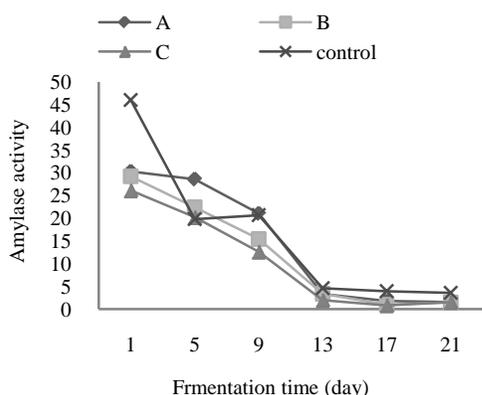


Fig. 5: The amylase activity of soy sauce in the fermentation

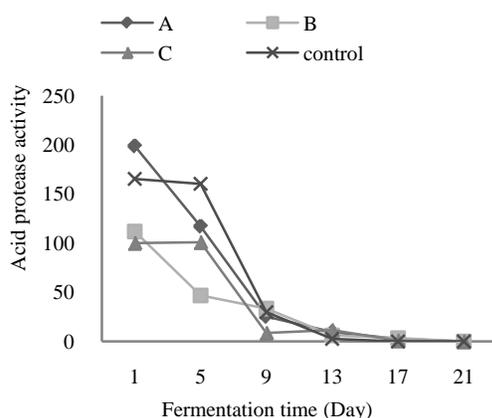


Fig. 6: The acid protease activity of soy sauce in the fermentation

soy sauce prepared with bittern had a higher content of reducing sugars than that the traditional one.

Changes of amino nitrogen: The contents of amino nitrogen (Fig. 4) were found to be lower in soy sauce

prepared with bittern than that traditional one and the less the bittern, the higher the content. The high protease activity promoting protein hydrolysis contributed to the rapid increase in the first 5 days, the accumulation of the various ingredients caused the decline of protease activity, thus the rate of the increase slowed down, or proteins that easy to be degraded had been degraded, protein itself could not easily be degraded needed more time to be degraded. Besides, Maillard reaction decreased the amino nitrogen

Changes of the enzyme activity: The fermentation of the soy sauce is using the enzyme to hydrolyze proteins and starch, forming a certain amount of substances such as amino acids, sugars, given unique flavor. The protease and amylase activity is on a declining curve with the decomposition of protein and starch.

It can be seen from the Fig. 5 that the amylase activity of the traditional one decreased rapidly in the first 5 days. Though not as fast as the control, the amylase activity of bittern soy sauce was also decreased significantly. As the fermentation progressed, their amylase activity slowed down gradually, but existed in the whole fermentation process. The soy sauce placing the whole salt with bittern showed lowest amylase activity under the influence of the acidity.

The Acid Protease (AIP) activity of the Koji was 147.3212 U/g. the changes of the protease activity in the fermentation can be seen in the Fig. 6. The protease activity of the soy sauce decreased rapidly in the first 9 days and it was very weak in the last 5 days. As can be seen from the results of the experimental group and control group, the pH had a greater impact on the enzyme activity in the fermentation process. The pH of the mustard tuber was 3.54, much lower than salt, so adding bittern into the raw material will affect the whole fermentation environment.

CONCLUSION

For the influence of the acidity of the mustard tuber bittern, the biochemistry properties of the bittern soy sauce were affected to some extent. But it can be seen from the result that the fermentation is feasible so that the new bittern soy sauce can be applied to develop a wide variety of condiments in the future.

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