

Full Length Research Paper

Biochemical changes in low-salt fermentation of solid-state soy sauce

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Low-salt solid-state fermentation soy sauce was prepared with defatted soy bean and wheat bran. Biochemical changes during the aging of the soy sauce mash were investigated. Results show that after a 15-day aging period, the contents of total nitrogen, formol titration nitrogen, free amino acids, reducing sugar, total sugar and the brown color were increased. However pH was decreased during the fermentation period. Furthermore contents of free amino acids in low-salt solid-state fermentation soy sauce fluctuated during the fermentation period with most of the free amino acids increased. The analysis of free amino acid composition shows that the contents of glutamic acid, aspartic acid, alanine and leucine were higher than other amino acids. Therefore it means that these amino acids may contribute to the taste and flavor of low-salt solid-state fermentation soy sauce. Analyzing the biochemical change in the fermented process of soy sauce is helpful to find out the shortcoming of low-salt solid-state fermented soy sauce. It is of benefit in improving the quality of low-salt solid-state fermented soy sauce.

Key words: Chemical composition, fermentation processing, free amino acid, low-salt solid-state fermentation soy sauce.

INTRODUCTION

Soy sauce is a traditional fermented condiment commonly consumed by the people in China, Korea and Japan (Mongkolwai, 1997). Soy sauce koji is traditionally prepared by growing the koji mold such as *Aspergillus oryzae* on the raw material containing a mixture of steam-cooked defatted soybean and wheat bran. Soy sauce mash obtained by mixing the finished koji with brine solution was then subjected to various periods of aging (Ling and Chou, 1996; Nan-Wei Su, et al., 2005).

There are two fermentation soy sauces in China. The main difference between them is the aging time. In China, the traditional aging period is about 180 days, the soy sauce fermented by this method is called high salt liquid-state fermentation soy sauce, and the brine solution concentration is about 20%. The other is called low-salt solid-state fermentation soy sauce, the period of this soy sauce is about 15 days, and the brine solution concentration is about 15%.

Compared with the high salt liquidation soy sauce has the following advantages: shorter production cycle and lower production cost; furthermore chemical analysis satisfies the national standards of China. But the taste of high salt liquid-state fermentation soy sauce is a little better than low-salt solid-state fermentation soy sauce. However, there are lots of papers concerning the soy sauce fermented and enzyme activities in soy sauce fermented (Su, 1980; Chou and Ling, 1998; Nelson, 1944), while others focused on the taste of fermented soybean food (Kim and Lee, 2003; Han et al., 2004). This paper reports on the taste and biochemical changes of low-salt solid-state fermented soy sauce. In order to find the characters of biochemical changes and further improve the flavor of low-salt solid-state fermented soy sauce, we studied some bio-chemical changes during the aging of soy sauce mashes of low-salt solid-state fermentation soy sauce.

MATERIALS AND METHODS

Preparation of seed koji

The substrate was composed of defatted soybean and wheat bran

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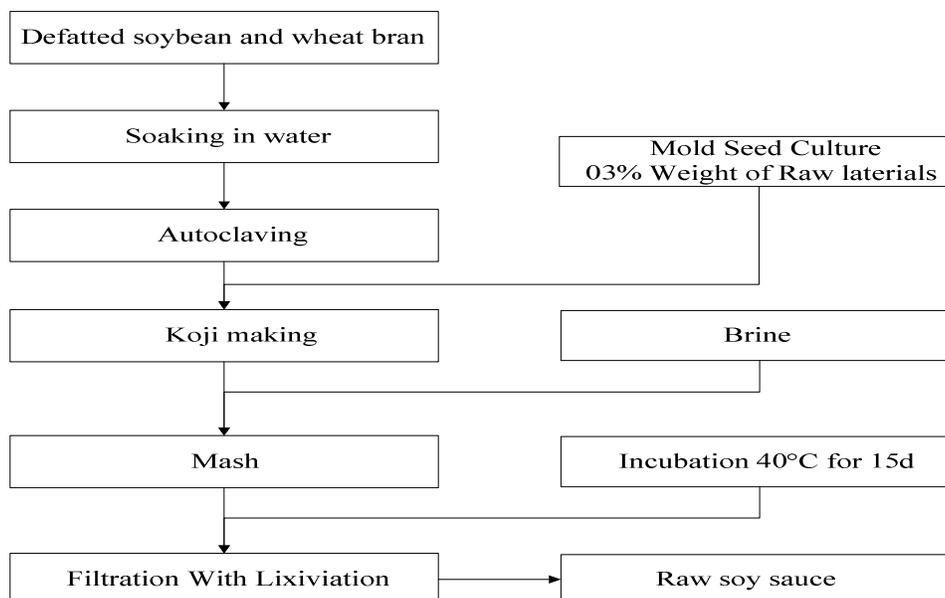


Figure 1. Procedure for manufacturing soy sauce.

at a ratio of 1:3 (w/w). To prepare seed koji, the substrate with 60% water was first cooked at 121°C for 30 min, then cooled to room temperature and inoculated with spores of *A. oryzae* and incubated at 30°C for 3 days.

Preparation of soy sauce koji

Essentially, the procedures and conditions employed in the soy sauce factories in China were followed to prepare the soy sauce koji and to age the soy sauce mash. Raw materials were thoroughly mixed with seed koji (approximately 10^8 spores g^{-1}) at a ratio of 1:1000 (w/w) and were placed on koji trays (diameter: 85 mm). They were kept in temperature of $30 \pm 2^\circ C$ for 44 h. After 24 h of incubation, the cultures were stirred and the temperature was lowered to 25°C for the rest of the incubation time.

Aging of soy sauce mash

The finished koji was divided into two parts. Each part of the prepared koji was then mixed with brine solution (14%) at a ratio of 1:1.7 (w/v) and was placed in individual cylindrical mash tanks (60 L). During the period of aging, the temperature was controlled at 45°C. The mashes were stirred for 10 min once every day for the first three days. The mashes were then covered with salt (thickness: 2 cm) for the remaining period. The total aging period was 15 days. The final concentration of NaCl in soy sauce is about 15%. The procedure of soy sauce fermented is shown in Figure 1.

Chemical analysis

At various time intervals during the aging period, samples of 100 g soy sauce mash was taken from each of the mash tanks containing the same type of koji. They were individually stirred and centrifuged at 8 000 rpm for 30 min. The supernatants were filtrated through Whatman no. 3 paper. The filtrate, regarded as raw soy sauce, was placed in brown bottles and kept at 4°C until analysis. For each analysis, three replicates tests were performed and all results presented are the average of duplicates experiments. Contents of

total nitrogen and formol titration nitrogen in raw soy sauce were determined according to methods described by AOAC (1994). The method described by Nelson (1944) was used to determine the reducing sugar content while pH was measured directly with a pH meter (Ishigami, 1965). Carbohydrate content was determined by the phenol-sulphuric acid method of Dubois and others (Dubois, 1956) in three replicates. The brown color intensity of soy sauce was determined by measuring the absorbance at 520 nm with a spectrophotometer according to Hashiba (1974). For determination of free amino acids, 1 ml samples was precipitated with 10% trichloroacetic acid (TCA) into 50 ml for 2 h at ambient temperature and then centrifuged at 10 000 rpm for 10 min to remove large peptides. The supernatant was filtered through 0.45 μm membrane. The sample solution was analyzed by an Agilent HP1100 HPLC (Agilent, USA) equipped with an Agilent Zorbax 80A Extend-C₁₈ column (150 \times 4.6 mm i.d., particle size 5 μm), an o-phthalaldehyde (OPA) forward-column derivatization autosampler, and a UV detector. The mobile phases were: A, 20 mmol sodium acetate (pH = 7.2) with 0.5% tetrahydrofuran; B, 20 mM sodium acetate (pH = 7.2)/ methanol/acetonitrile (1:2:2, by volume). The linear elution gradient was A:B (by volume) from 100:0 to 50:50 for 0 - 17 min, 50:50 to 0:100 for 17 - 20 min, and 100:0 for 20 - 24 min. The flow rate was 1.0 ml/min. The temperature was controlled at 40°C. The amino acids were detected at 338 nm except for proline, which was detected at 262 nm. Each amino acid was identified by comparing the samples with a standard (Sigma, USA) analyzed under the same conditions and quantified by the calibration curve of the authentic compound.

RESULTS AND DISCUSSION

During the fermentation of soy sauce, proteins in the raw materials are hydrolyzed into small molecular weight peptides, amino acids and ammonia by the proteases produced by *A. oryzae* (Whitaker, 1978). Nitrogen constituents are the important parameter used for grading the quality of soy sauce product. According to the Chinese

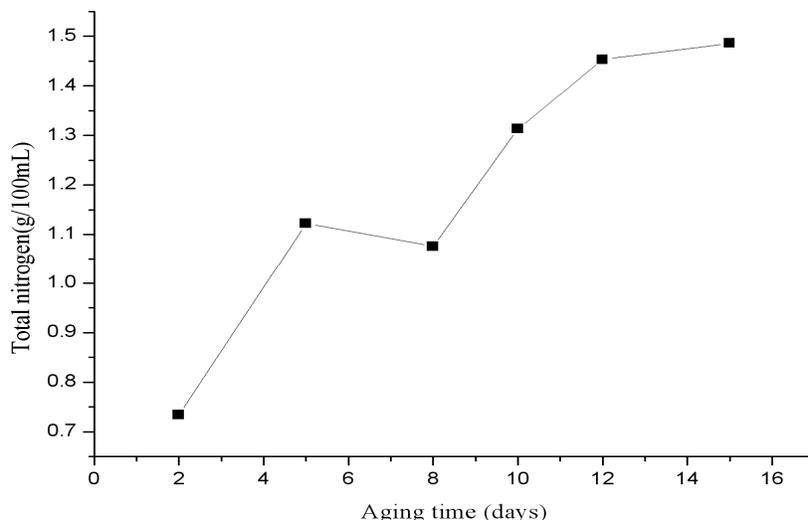


Figure 2. Content of total nitrogen in raw soy sauce at various periods of aging.

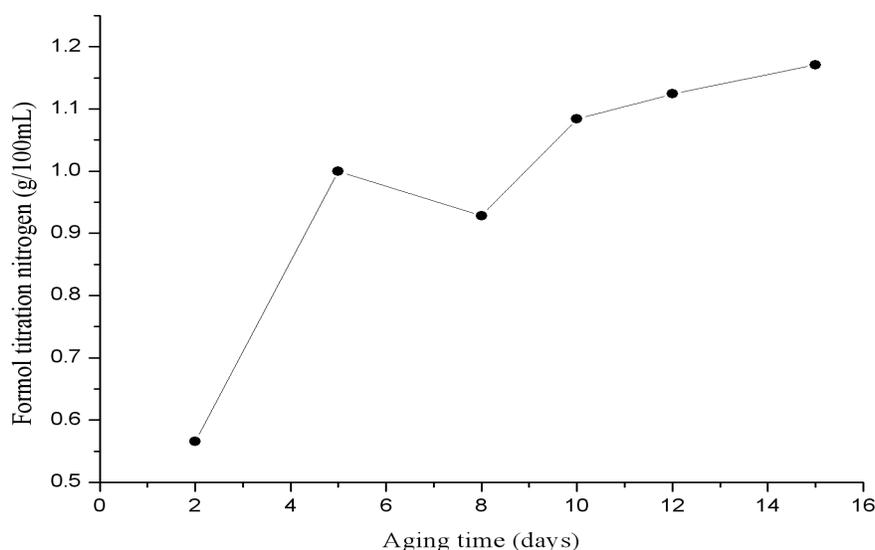


Figure 3. Content of formol titration nitrogen in raw soy sauce at various periods of aging.

National Standard (2000), Grade A soy sauce should contain total nitrogen and formol titration nitrogen of more than 1.4 and 0.70%, respectively. The contents of total nitrogen and formol titration nitrogen in raw soy sauce during the aging period are presented in Figures 2 and 3, respectively. It is shown that a rapid increase in these contents occurred during the initial period of aging days. At the end of aging, low-salt solid-state fermentation soy sauce contained 1.49% total nitrogen (Figure 2) and 1.12% formol titration nitrogen (Figure 3). Low-salt solid-state fermentation soy sauce can be classified as Grade A soy sauce according to the Chinese National Standard.

Figure 5 shows the contents of individual free amino acids and Figure 4 shows the total free amino acids in

low-salt solid-state fermentation soy sauce at various periods of aging. Although the contents of some individual free amino acids in low-salt solid-state fermentation soy sauce fluctuated during the aging process, most free amino acids increased as expected (Figure 5). Total free amino acids increase in the contents were noted at the 15-day aging period (Figure 4). These compounds are released by microbial action (mainly by the microbial enzymes), through the biochemical reactions that are taking place during fermentation. Among the free amino acids, glutamic acid, aspartic acid, alkaline, leucine, lysine and glycine showed a larger increase in low-salt solid-state fermentation soy sauce at the end of aging. The major free amino acids were found as glutamic acid,

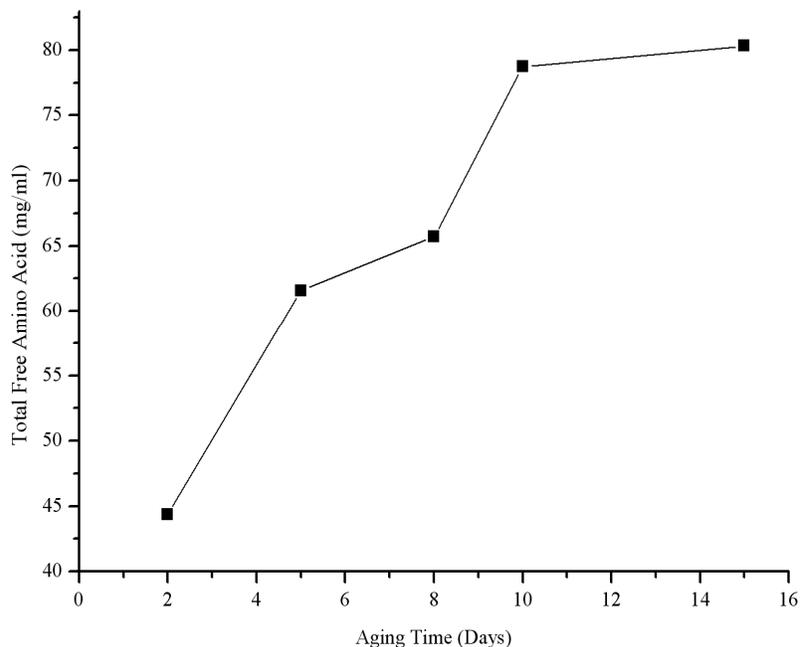


Figure 4. Content of total free amino acid in raw soy sauce at various periods of aging.

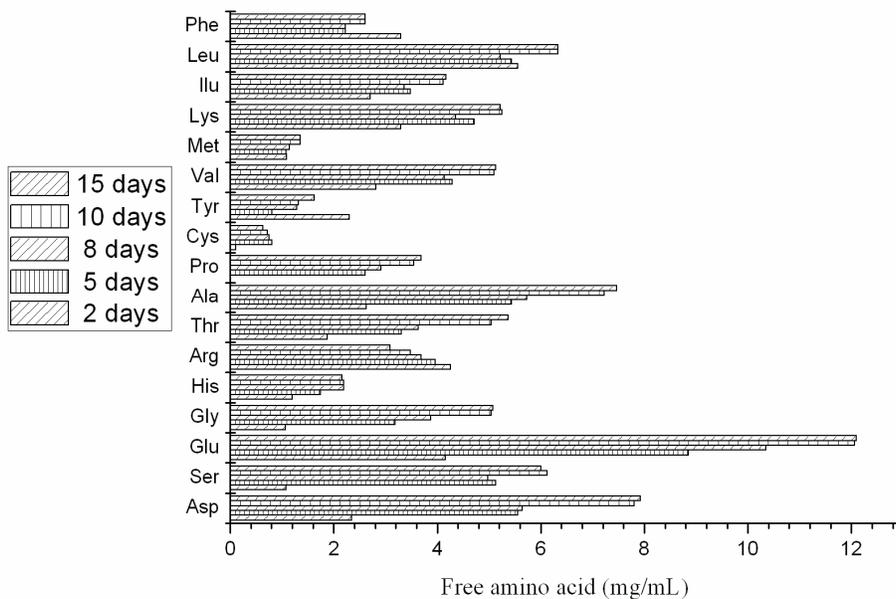


Figure 5. Composition of free amino acid in raw soy sauce at various periods of aging.

aspartic acid, and leucine throughout the whole fermentation period. These amino acids, especially glutamic acid, were regarded to be important contributors towards the flavor of soy sauce (Nishimura and Kato, 1988; Je et al., 2005; Sarkar et al., 1997). Among the free amino acids, arginine showed a clearly decline in low-salt solid-state fermentation soy sauce at the end of aging. The

increase of the content of leucine and the decrease of the content of arginine is considered to contribute to the debittering of soy sauce.

Figure 6 shows the changes in total sugar and Figure 7 shows the changes in reducing sugar content in low-salt solid-state fermentation soy sauce during aging period. The content of reducing sugar increased rapidly as the

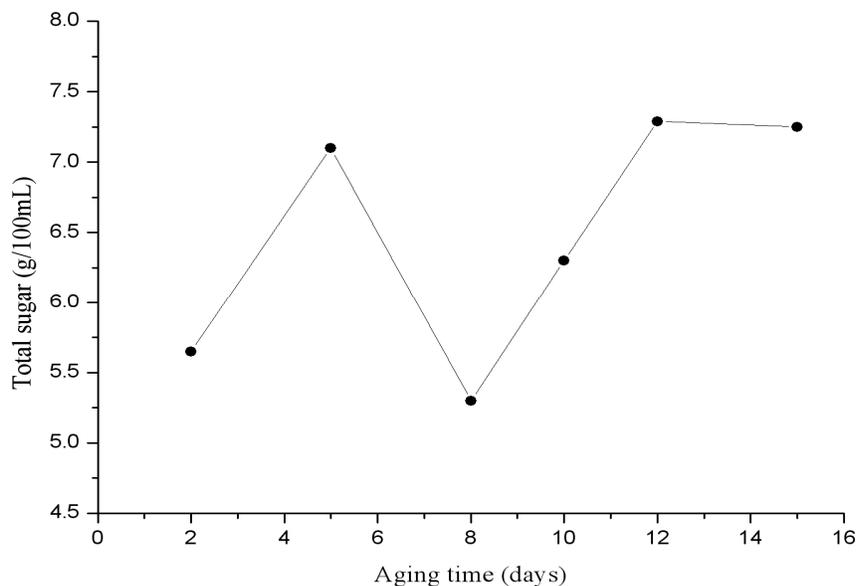


Figure 6. Changes of total sugar in raw soy sauce at various periods of aging.

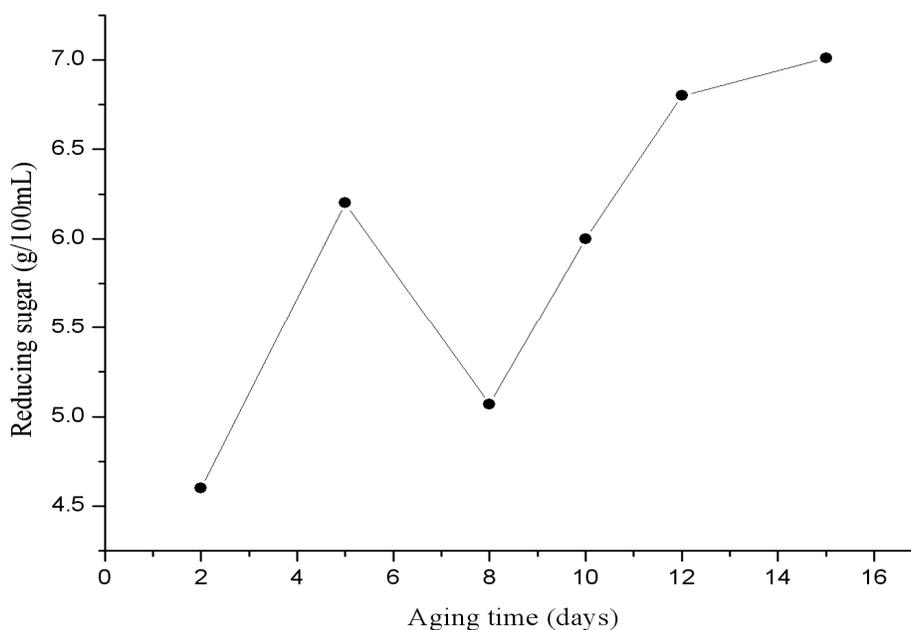


Figure 7. Content of reducing sugar in raw soy sauce at various periods of aging.

aging process began, reaching its maximum after 5 days and decreased thereafter. A similar phenomenon also occurred on the changes of total sugar that it increased rapidly as the aging process began, reaching their maximum after 5 days but decrease after 10 days from the beginning of the aging. The rapid increase in reducing sugar content during the initial aging period may be attributed to the action of fungal amylase activity. The rapid increase in total sugar content during the initial

aging period may be attributed to the dissolution of soluble polysaccharides. However, as aging proceeded, amylase activity decreased due to enzyme denaturation in brine solution (Chou et al., 1998). The other reason of the reducing sugar and total sugar decrease in aging period may be apparently due to the amino-carbonyl Maillard reaction.

The pH of low-salt solid-state fermentation soy sauce decreased during the days of aging (Figure 8). At the first

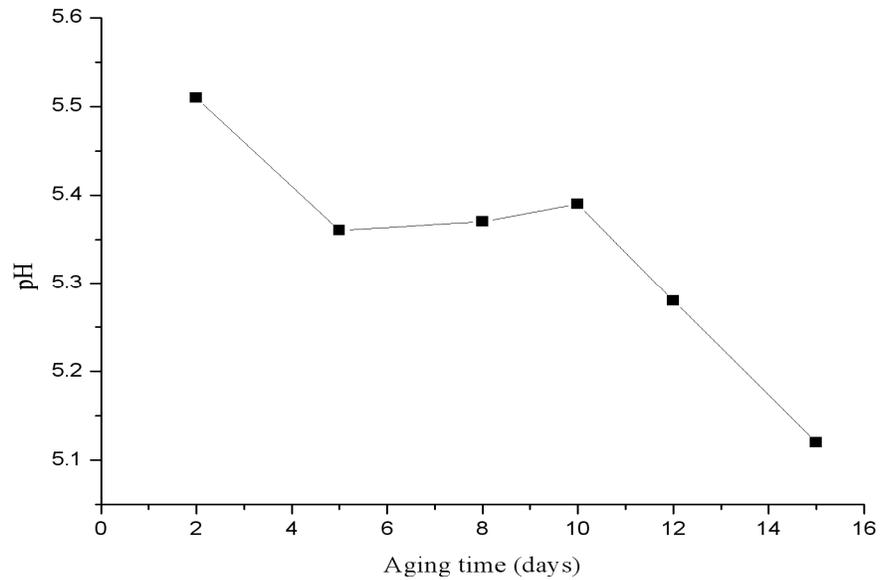


Figure 8. pH of raw soy sauce at various periods of aging.

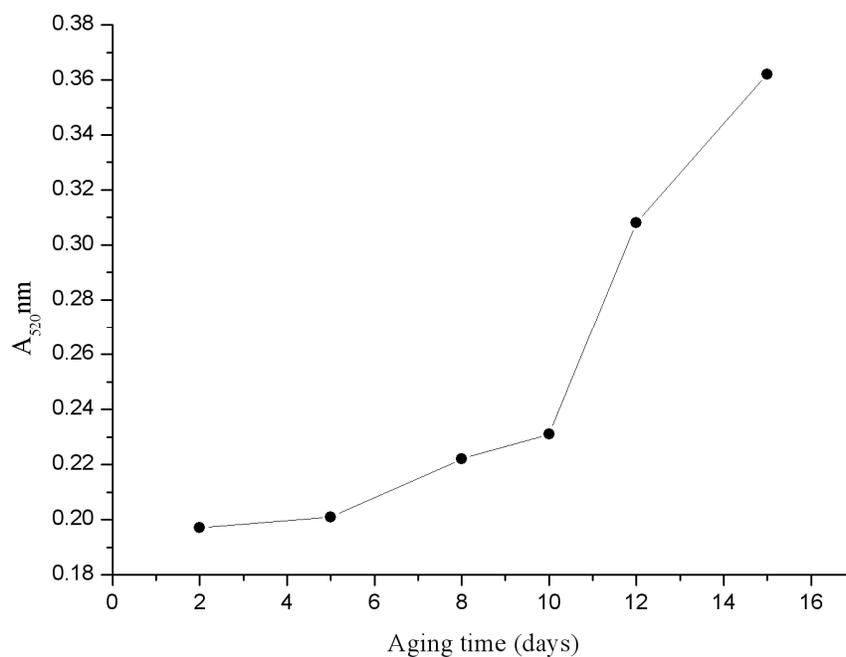


Figure 9. Browning of raw soy sauce during the aging.

5 days of aging, it decreased rapidly. But at the second 5 days, there was a little rise. Then the pH decreased rapidly from 5.39 to 5.12 at the end of the third 5 days. The decline of pH during the aging of mash may be attributed to autolysis of microbial cells, accumulation of free fatty acid, amino acids, and peptides containing carboxylic side chains as a result of hydrolysis of mash constituents, as well as the microbial fermentation of

carbohydrates (Shieh et al., 1982).

Extrusion of soy sauce raw material resulted in a deep brown color, apparently due to the amino-carbonyl Maillard reaction which occurred under the high pressure and high temperature conditions (Noguchi et al., 1982). The intensity of brown color in the low-salt solid-state fermentation soy sauce, determined at $A_{520 \text{ nm}}$, increased progressively as aging proceeded (Figure 9).

Oxidative browning reaction has been reported to occur in soy sauce during the aging process (Dubois et al., 1956).

As shown in Figure 8, the intensity of brown color increased slowly at the first 5 days, at the second 5 days it increased rapidly, at the third 5 days it increased more rapidly than ever.

Conclusion

In this paper, we analyzed the changes of chemical composition and amino acids of the low-salt solid-state fermentation soy sauce with different fermentation periods. In soy sauce fermented by *A. oryzae*, total nitrogen, formol titration nitrogen and amino acid contents indicated the hydrolysis of soybean proteins, and total sugar and reducing sugar contents indicated the hydrolysis of polysaccharide. Total nitrogen, formol titration nitrogen and amino acid contents were obviously increased at the end of the fermentation. However the contents of total sugar and reducing sugar increased at the beginning of fermentation, but decreased subsequently. Altogether the chemical analysis showed that the quality of low-salt solid-state fermented soy sauce could fulfill the Chinese national standard, and could reach Grade A. Free amino acids, such as glutamic acid, aspartic acid and alanine, were abundant and are recognized as being important in the tastes of low-salt solid-state fermentation soy sauce. Therefore, these results suggested that the amino acid content elevated could provide valuable information on optimal low-salt solid-state fermentation soy sauce. Improving the flavor compound in low-salt solid-state fermented soy sauce is more important in improving the quality compared with the high-salt liquid-state fermented soy sauce.

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