

Full Length Research Paper

# Biochemical changes in low-salt solid-state fermented 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 showed 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 total free amino acids in low-salt solid-state fermentation soy sauce fluctuated during the fermentation period, and in most periods, it was 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. The biochemical changes characters were due to enzyme activities, solution balance and reaction balance, and biochemical changes contributed to the improvement of the flavor of low-salt solid-state fermented soy sauce. Analyzing the biochemical change in the fermented process of soy sauce is helpful in finding out the shortcoming of low-salt solid-state fermented soy sauce.**

**Key words:** Low-salt solid-state fermented soy sauce, biochemical changes, fermentation period, chemical composition.

## INTRODUCTION

Soy sauce is a traditional fermented condiment commonly consumed by the people in China, Korea and Japan (Luo et al., 2009). 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 is then subjected to various periods of aging (Ling and Chou, 1996; 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 one is called low-salt solid-state fermentation soy sauce, the aging period of this soy sauce is about 15 days, and the brine solution concentration is about 15%. In comparison with the high salt liquid-state fermentation soy sauce, low-salt solid-state fermentation soy sauce has the following advantages: shorter production cycle and lower production cost; furthermore, chemical analysis satisfies the national

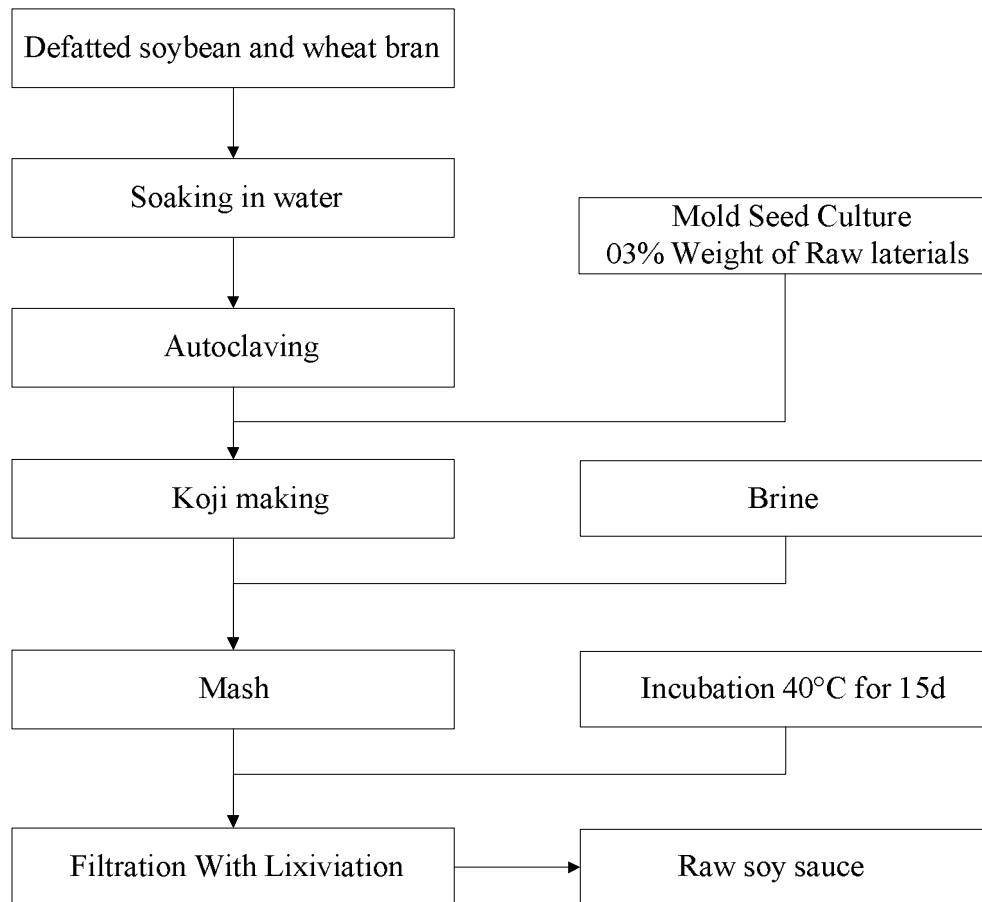
standards of China. But the taste of high salt liquid-state fermentation soy sauce is a little better than low-salt solid-state fermentation soy sauce. However, there are lot of papers concerning the soy sauce fermented and enzyme activities in soy sauce fermented (Su, 1980; Chou et al., 1998; Nelson, 1944); some were focused on the taste of fermented soybean food (Kim and Lee, 2003; Han et al., 2004) while others focus 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, some biochemical changes during the aging of soy sauce mashes of low-salt solid-state fermentation soy sauce was studied.

## MATERIALS AND METHODS

### Preparation of koji seed

The substrate was composed of defatted soybean and wheat bran at a ratio of 1:3 (w/w). To prepare koji seed, the substrate with 60%



**Figure 1.** Procedure for manufacturing soy sauce.

water was first cooked at 121°C for 30 min, then cooled to room temperature and inoculated with spores of *Aspergillus 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 know the age of the soy sauce mash. The 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

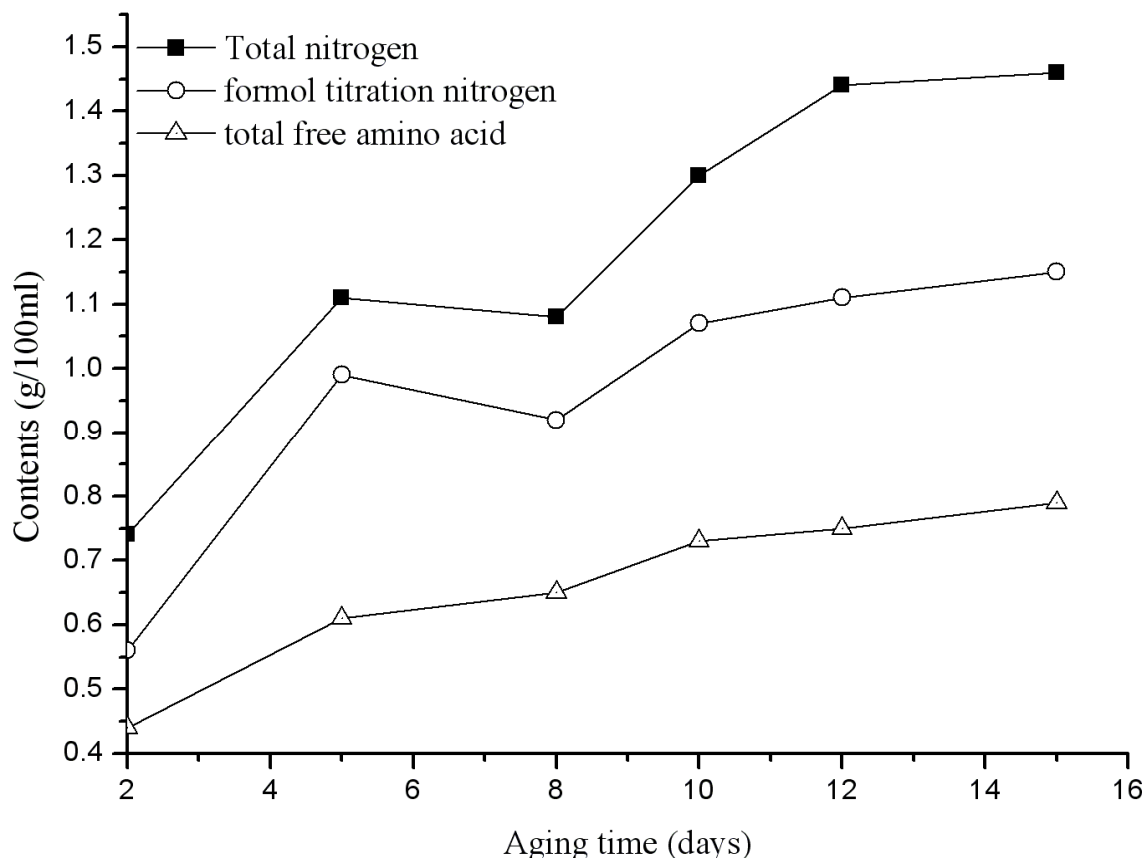
The finished koji was divided into two parts. Each part of the prepared koji was then mixed with brine solution (14 %) at a ratio of 1:1.7 (w/v) and was placed in individual cylindrical mash tanks (60 L). During the period of aging, the temperature was controlled at 45°C. The mashes were stirred for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

#### Chemical analysis

At various time intervals, during the aging period, samples of 100 g soy sauce mash were taken from each of the mash tanks containing the same type of koji. They were stirred individually 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 et al. (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 sample 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  $\mu m$  membrane. The sample solution prepared in 20  $\mu l$  was analyzed by an Agilent HP1100 HPLC (Agilent, USA) equipped with an Agilent Zorbax 80A Extend-C<sub>18</sub> column (150  $\times$  4.6 mm i.d., particle size 5  $\mu m$ ), an o-phthalaldehyde (OPA) forward-column derivatization autosampler and a UV detector. The mobile phases



**Figure 2.** Content of total nitrogen, formol titration nitrogen and total free amino acid in raw soy sauce with various periods of aging.

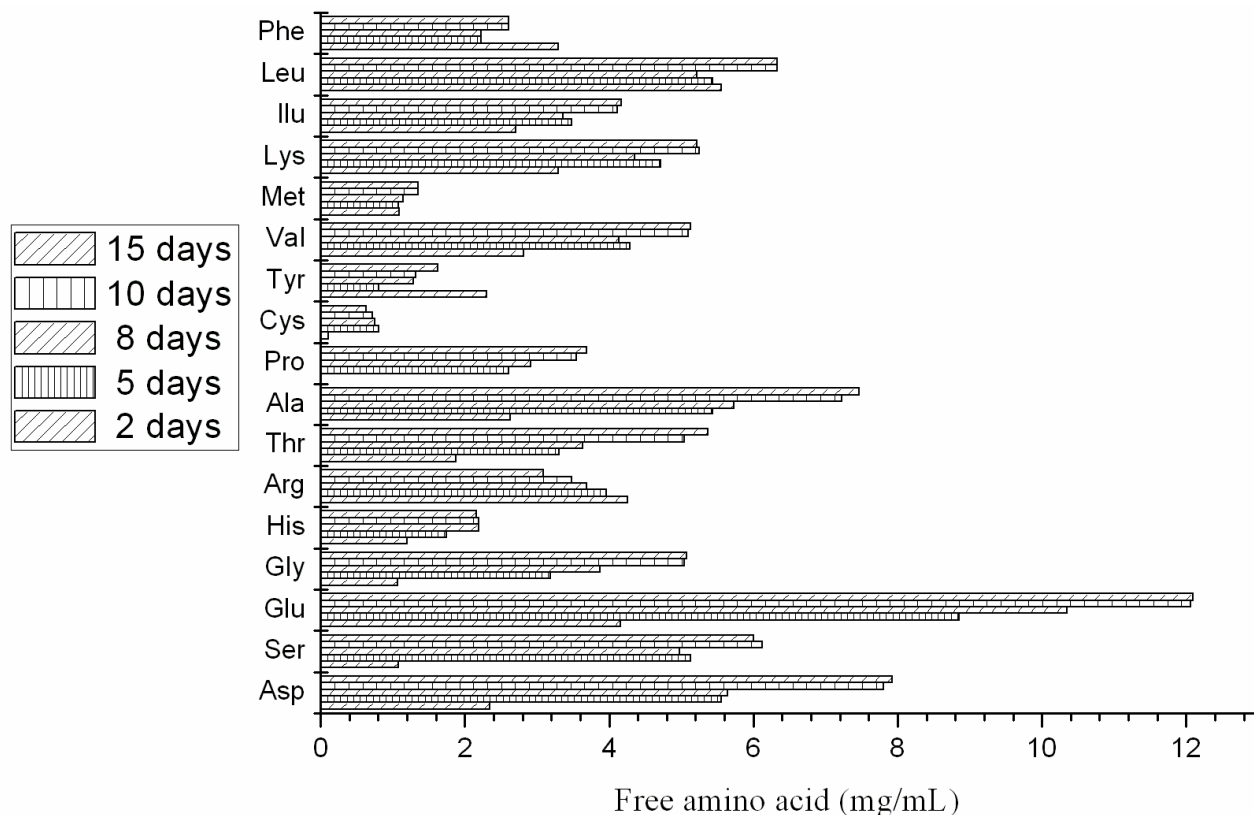
were: A, 20 mmol sodium acetate (pH = 7.2) with 0.5% tetrahydrofuran; B, 20 mmol sodium acetate (pH = 7.2)/methanol/acetonitrile (1:2:2, by volume). The linear elution gradient was A:B (by volume) from 100:0 to 50:50 for 0 - 17 min, 50:50 to 0:100 for 17 - 20 min, and 100:0 for 20 - 24 min. The flow rate was 1.0 ml/min. The temperature was controlled at 4°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 were hydrolyzed into small molecular weight peptides, amino acids and ammonia by the proteases produced by *A. oryzae* (Whitaker, 1978). Nitrogen constituents are important parameters used for grading the quality of soy sauce product. According to the Chinese 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 Figure 2. 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 and 1.12% formol titration nitrogen (Figure 2). At day 8 during the aging period, the total nitrogen, formol titration nitrogen and total free amino acid decreased obviously because of amino-carbonyl Maillard reaction. At this period, the soluble nitrogen was consumed rapidly because the reaction rate is higher than the solution rate. After that, enzyme hydrolysis increased the solution rate, so the contents of nitrogen and amino acid in soy sauce were increased. Low-salt solid-state fermentation soy sauce can be classified as Grade A soy sauce according to the Chinese National Standard.

Figure 3 shows the contents of individual free amino acids and Figure 2 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 were increased as expected (Figure 3). Total free amino acids increase in the contents was noted at the 15-day aging period (Figure 2). These compounds are released by microbial action (mainly by the microbial



**Figure 3.** Composition of free amino acid in raw soy sauce with various periods of aging.

enzymes) through the biochemical reactions that takes place during fermentation. Among the free amino acids, contents of 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, aspartic acid, and leucine throughout the whole fermentation period. These amino acids, especially glutamic acid, were regarded as 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, contents of arginine showed a clear decline in low-salt solid-state fermentation soy sauce at the end of aging. The increase in the content of leucine and the decrease in the content of arginine are considered as contributing factors to the debittering of soy sauce.

Figure 4 shows the changes in total sugar and reducing sugar content in low-salt solid-state fermentation soy sauce during aging period. The contents of reducing sugar increased rapidly as the aging process began, reaching its maxima after 5 days and decreasing thereafter. A similar phenomenon has also occurred in the content change of total sugar that increased rapidly as the aging process began, reaching their maxima after 5 days but decreasing 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

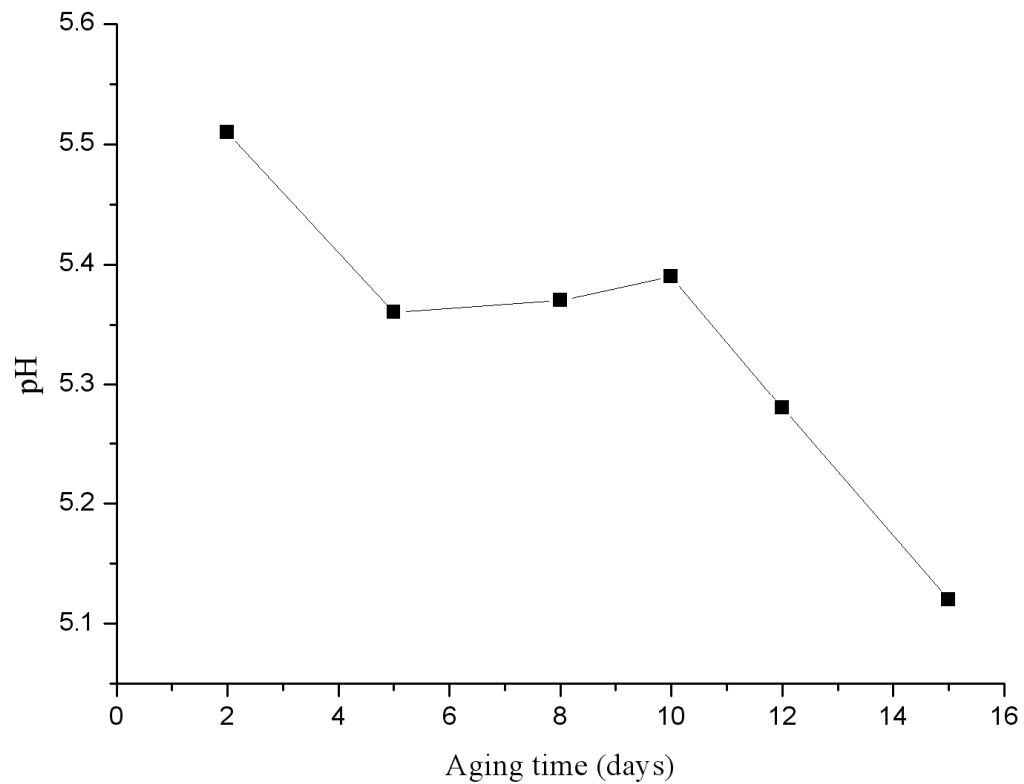
fungal amylase activity. The rapid increase in total sugar content during the initial aging period may be attributed to the dissolution of soluble polysaccharides. However, as aging proceeded, amylase activity was decreased due to enzyme denaturation in brine solution (Chou et al., 1998). The other reason for the reduced content of sugar and decreased total sugar in aging period is 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 5). At the first 5 days of aging, it decreased rapidly. But at the second 5 days, it had 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).

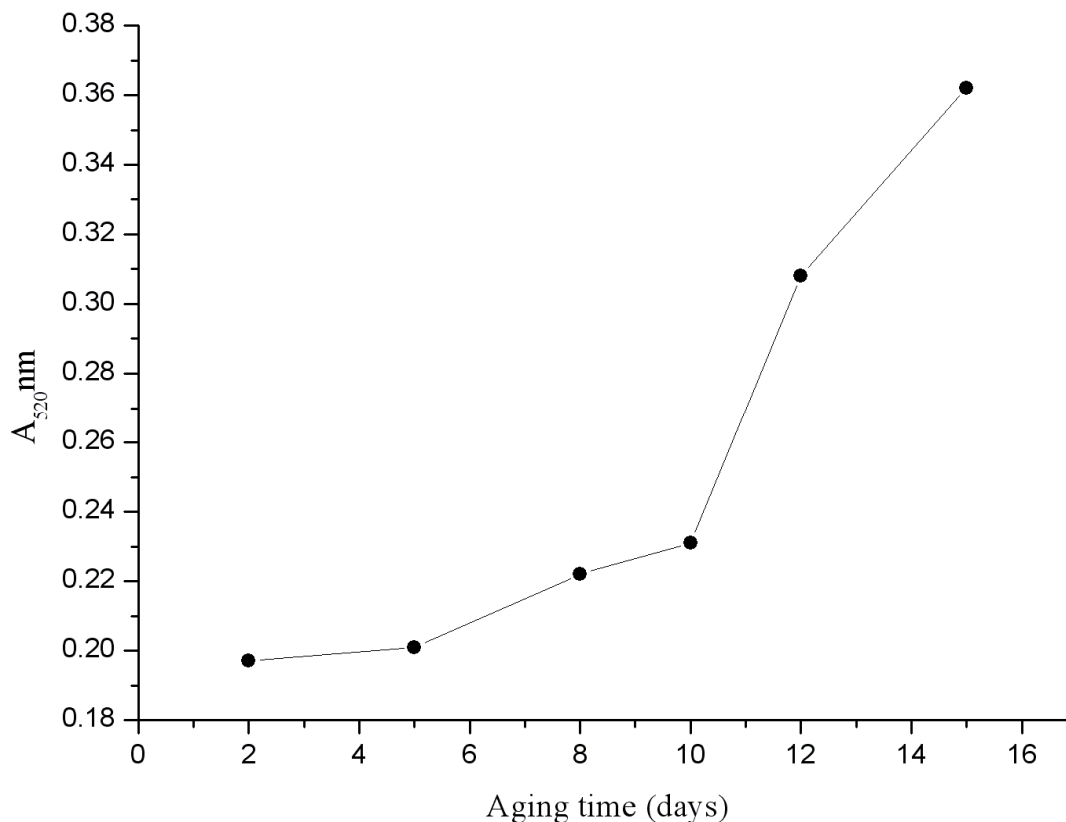
Extrusion of soy sauce raw material resulted in a deep brown color, apparently due to the amino-carbonyl Maillard reaction which occurred under the high pressure and high temperature conditions (Noguchi et al., 1982). The intensity of brown color in the low-salt solid-state fermentation soy sauce, determined at A520 nm, increased progressively as aging proceeded (Figure 6). Oxidative browning reaction has been reported to occur in soy



**Figure 4.** Changes of total sugar and reducing sugar in raw soy sauce with various periods of aging.



**Figure 5.** pH of raw soy sauce with various periods of aging.



**Figure 6.** Browning of raw soy sauce during the aging period.

sauce during the aging process (Dubois et al. 1956). As shown in Figure 6, 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, the changes of chemical composition and amino acids of the low-salt solid-state fermentation soy sauce with different fermentation periods was analyzed. In the process of soy sauce fermentation using *A. oryzae*, total nitrogen, formol titration nitrogen and amino acid contents, there was hydrolysis of soybean proteins, while the total sugar and reducing sugar contents showed the hydrolysis of polysaccharide. Total nitrogen, formol titration nitrogen and amino acid contents were increased obviously at the end of the fermentation, though it has some decreases at day 8 of aging period. Also, with the characters of biochemical changes, 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, it could also reach the grade A. Free amino acids such as glutamic acid, aspartic acid and

alanine were abundant and recognized as being important in the taste of low-salt solid-state fermentation soy sauce. Therefore, these results suggested that the biochemical changes characters were due to enzyme activities, solution balance and reaction balance, and biochemical changes contributes to improvement of the flavor of low-salt solid-state fermented soy sauce. Improving the flavor compound in low-salt solid-state fermented soy sauce is more important in improving the quality when compared to the high-salt liquid-state fermented soy sauce.

## REFERENCES

- Chinese National Standard, Fermentation soy sauce. GB 18186-2000, (2000).
- Chou C-C, Hwang GR, Ho FM (1998). Changes of microbial flora and enzyme activity during the aging of Tou-Pan-Chiang, a Chinese fermented condiment. *J. Ferment. Technol.* 66: 473-478.
- Chou C-C, Ling M-Y (1998). Biochemical changes in soy sauce prepared with extruded and traditional raw materials, *Food Res. Int.* 31: 487-492.
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956). New colorimetric methods of sugar analysis VII. The phenol-sulfuric acid reaction for carbohydrate. *Anal. Chem.* 28: 350-356.
- Han B-Z, Rombouts FM, Nout MJR (2004). Amino acid profiles of sufu, a Chinese fermented soybean food, *J. Food Comp. Anal.* 17: 689-698.
- Hashiba H (1974). Effect of ageing on the oxidative browning of

- sugar amino acid model systems. *Agric. Biol. Chem.* 38: 551-555.
- Ishigami Y, Ishikawa H, Ueda R, Taketsuru H (1965). Studies on the changes in microflora and its metabolisms during Koji-making process in soy sauce brewing. *J. Ferment. Technol.* 43: 165-173.
- Je J-Y, Park P-J, Jung W-K, Kim S-K (2005). Amino acid changes in fermented oyster (*Crassostrea gigas*) sauce with different fermentation periods. *Food Chem.* 91: 15-18.
- Kim S-H, Lee K-A (2003). Evaluation of taste compounds in water-soluble extract of a doenjang (soybean paste). *Food Chem.* 83: 339-342.
- Ling MY, Chou CC (1996). Biochemical changes during the preparation of soy sauce koji with extruded and traditional raw materials. *Int. J. Food Sci. Technol.* 31: 511-517.
- Luo JQ, Ding LH, Chen XR, Wan YH (2009). Desalination of soy sauce by nanofiltration. *Sep. Purif. Technol.* 66: 429-437.
- Nelson NA (1944). Photometric adaptation of the Somogi method for the determination of glucose. *J. Biol. Chem.* 15: 375-381.
- Nishimura T, Kato H (1988). Taste of free amino acids and peptides. *Food Rev. Int.* 4: 175-194.
- Noguchi A, Mossor K, Aymord C, Jeunik J, Cheftel JC (1982). Maillard reaction during extrusion cooking of protein enriched biscuits. *Lebens Wiss Technol.* 15:105-108.
- Official Methods of Analysis of the Association of Official Analytical Chemists, 14th ed. AOAC International, Gaithersburg, USA. (1994).
- Sarkar PK, Jones LJ, Craven GS, Somerset SM, Palmer C (1997). Amino acid profiles of kinema, a soybean fermented Food. *Food Chem.* 59: 69-15.
- Shieh YSC, Beuchat LR, Worthington RE, Phillips RD (1982). Physical and chemical changes in fermented peanut and soybean pastes containing koji prepared using *Aspergillus oryzae* and *Rhizopus oligosporous*. *J. Food Sci.* 47: 523-529.
- Su N-W, Wang M-L, Kwok K-F, Lee M-H (2005). Effects of temperature and sodium chloride concentration on the activities of proteases and amylases in soy sauce koji. *J. Agric. Food Chem.* 53: 1521-1525.
- Su YC (1980). Traditional fermented foods in Taiwan. In Proceedings of the Oriental Fermented Foods, Food Industry Research and Development Institute, Hsinchu, Taiwan, Republic of China. pp. 15-30.
- Whitaker JR (1978). Biochemical changes occurring during the fermentation of high-protein foods. *Food Technol.* 32: 175-180.