African Journal of Biotechnology Vol. 9(5), pp. 702-706, 1 February, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Effect of temperature on moromi fermentation of soy sauce with intermittent aeration

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Accepted 14 December, 2009

Soy sauce has been widely used as one of the main seasoning agents in Asian countries. Soy sauce is produced by two-steps fermentation processes, namely koji fermentation and moromi fermentation. In this study, different temperatures (25, 35 and 45°C) for moromi fermentation in bioreactor were investigated for understanding their influences on soya sauce quality, in terms of pH variations, ethanol concentrations and total nitrogen contents in raw soy sauce during moromi fermentation. It was learned that as the aging of moromi took place, the pH level was decreased from pH 7 to 4.88. Also, the soy sauce had lower concentration of ethanol when higher temperature was used in moromi fermentation but the difference of temperature did not show significantly effect on total nitrogen content in soy sauce. This study indicated that the temperature used in the moromi fermentation, coupled with intermittent aeration, imposed significant effects on soy sauce aging and quality. Higher fermentation temperature of 45°C enhanced the aging of soy sauce, accompanying with lower contents of ethanol and higher pH level in soy sauce. However, the total nitrogen content in the soy sauce was not significantly influenced by the fermentation temperature.

Key words: *Aspergillus oryzae*, bioreactor, moromi, soy sauce, temperature.

INTRODUCTION

Soy sauce is a traditional fermented food in Malaysia that has been practiced long time ago. Traditionally, soy sauce has been used in Japan and several oriental countries and is presently used as a liquid seasoning in cooking worldwide (Kataoka, 2005; Yokotsuka, 1986). Soy sauces are the main condiments for foods and table-top seasoning in the most of the Asian countries.

In Malaysia, there are so many varieties of soy sauces in the market such as dark soy sauce, light soy sauces, 'Kicap lemak manis', 'Kicap lemak masin' and others. Soy sauce in Malaysia is produced in small scale cottage industries. This production is mainly performed in a conventional method as compared to the Japanese production method using high technology machines for improving the tastes. Soy sauce in Malaysia is naturally brewed by two step fermentation processes, namely, koji fermentation and moromi fermentations. The koji fermentation process involves the mixtures of soybean and wheat flour with the inoculation of *Aspergillus oryzae*. During koji fermentation, the addition of *A. oryzae* excretes protease, amylase and other enzymes. These enzymes will hydrolyze the raw materials into simpler forms. Proteolytic enzymes will convert soy beans proteins into peptides and amino acids while amylase enzymes will hydrolyze starch into simple sugars. The hydrolyzed nutrients will be utilized by the yeast and bacteria in moromi stage.

In Malaysia, the moromi fermentation process is usually carried out in closed tanks that are kept under the sun for 3 - 4 months. In this process, there are three important

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types of microorganisms, which play crucial roles for a good soy sauce production. The simpler sugars from koji fermentation are mainly metabolized into lactic acid and acetic acid by *Pediococcus halophilus* (Iwasaki et al., 1993). The natural presence of yeast in the environment, namely *Zygosaccharomyces rouxii* and *Candida* species, will convert the remaining sugars to ethanol and a number of minor flavor compounds, respectively (Sasaki and Nunomura, 2003). During moromi fermentation, ethanol is being processed by *Z. rouxii* under aerobic and anaerobic condition (Hamada et al., 1989). Ethanol concentration is directly proportional to the increasing numbers in yeast cells during the moromi fermentation (Röling et al., 1996). On the other hand, *Candida* species are important for the development of aroma in soy sauce by producing phenolic compounds such as 4-ethylguaiacol (Hamada et al., 1990). For a good quality of soy sauce, it should contain 1.0 - 1.65% total nitrogen (w/v), 2.0 - 2.5% ethanol and 17 - 19% sodium chloride (w/v) with 45% of the total nitrogen being simple peptides and another 45%, amino acids (Luh, 1995).

Fermentation temperature is an important factor in defining the aging and quality of the soy sauce. In normal production of soy sauce, the fermentation temperature is usually dependent on the surrounding temperature. However, it was suggested that for better aging of soy sauce mash, the temperature for the soy sauce production were maintained at 15°C during the first month of fermentation and then the fermentation temperature was (gradually) raised to 30°C (Chou and Ling, 1998; Iwasaki et al., 1993). Later, Jansen et al. (2003) found that the production of fusel alcohols (which are important flavor compounds in the soy sauce) by *Z. rouxii* was also dependant on the fermentation temperature.

Aeration could be considered as one of the factors that would also influence the soy sauce production. Earlier, Beatrice Foods Co. (1972) reported that intermittent aeration could actually accelerate the maturity of soy sauce. Hamada et al. (1989) found that the soy sauce fermented by supplying air (0.02 vvm) had a higher content of aroma components. Later, Hamada et al. (1990) proposed the supply of air to fermentation broth was necessary for long time production of 4-ethylguaiacol in soy sauce fermentation. When lower aeration was supplied to the fermentation broth, the changes and production of flavor in soy sauce was very slow, accompanying by unripe flavor (Kim et al., 1996).

In the present study, the moromi fermentation was conducted in bioreactor with a maximum working volume of 6 L. The bioreactor system was equipped with aeration system and temperature sensor. The main aim of this study was to investigate the effect of temperature in the range of 25 to 45°C on the batch fermentation of soy sauce with intermittent aeration for 10 min at interval of three days. The quality of the soy sauce would be measured based upon the pH variations, ethanol concentrations and total nitrogen contents in raw soy sauce during moromi fermentation.

MATERIALS AND METHODS

Koji fermentation

For koji production, soy beans were first soaked in water for 10 h. Then, the soy beans were cooked by autoclave for 20 min at 121°C. The cooked soy beans were cooled to room temperature. The cooked soy beans were thoroughly mixed with wheat flour at a ratio of 3:1. Then, *A. oryzae* in powder form was inoculated at 0.1% of the cooked soybeans and spread evenly onto the mixture of beans and flour. They were placed on the perforated trays (30 x 24 x 2.5 cm). These trays were kept in a koji fermenter, which was equipped with a fan blower that sent wet air to the fermenter (Indoh et al., 2006). The koji was incubated for 72 h at temperature of 30°C throughout the experiment.

Moromi fermentation

The matured koji were equally divided and transferred separately to the bioreactor. For each bioreactor, the matured koji was mixed with a brine solution (20% w/v) at a ratio of 1:3. Temperature for each bioreactor was maintained differently, namely at 25, 35 and 45°C, while the control was kept separately from the bioreactors under ambient temperature (26 - 30°C). The temperatures in bioreactors (except the control) were maintained constantly throughout the experiment by jacketed vessel.

Also, intermittent aeration for 10 min at interval of three days was applied during the fermentation process. Aeration is required by the yeast in soy sauce production, in which case the yeasts are strict aerobes and need oxygen for their growth. When aeration is applied, in the early stage, it will retard the growth of salt tolerant lactobacilli and wild yeasts (O'Toole, 1997; Sasaki and Nunomura, 1993).

No agitation was introduced in this study because the organoleptic properties of soy beans in moromi stage were preferred to be maintained as a whole beans until the end of the experiment for reducing the problem in filtration of raw soy sauce.

Sampling and analytical methods

About 20 mL of sample was collected after the aeration took place. The sample was filtered through a muslin cloth and then under vacuum through a Whatman No. 4 filter paper (20-25 µm). The filtrate, regarded as raw soy sauce, was kept at 4°C for further analysis.
The pH of the soy sauce samples could be directly measured by using pH meter (HI 251, Hanna Instrument) (Ishigami et al., 1965). The total nitrogen contents in the soy sauce samples were analyzed by using Kjedhal Distillation Method while the ethanol contents were determined by using Gas Chromatography (GC-2010, Shimadzu). All data were reported as the mean of three independent trials.

RESULTS AND DISCUSSION

The fermentation of soy sauce production was carried out for 36 days. Moromi fermentation was carried out in three different fermentation temperatures, namely 25, 35 and

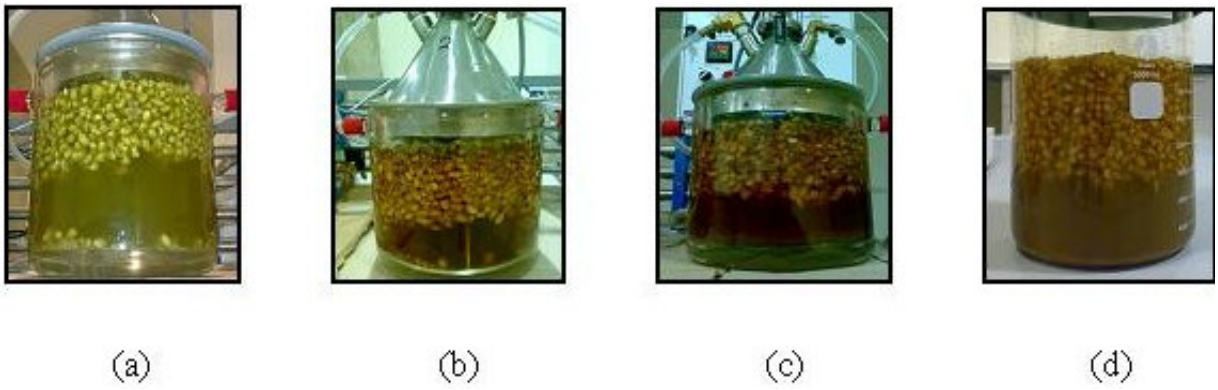

Figure 1. The color of the brine at (a) 25, (b) 35, (c) 45℃ and (d) room temperature (as control) after one day of moromi fermentation.

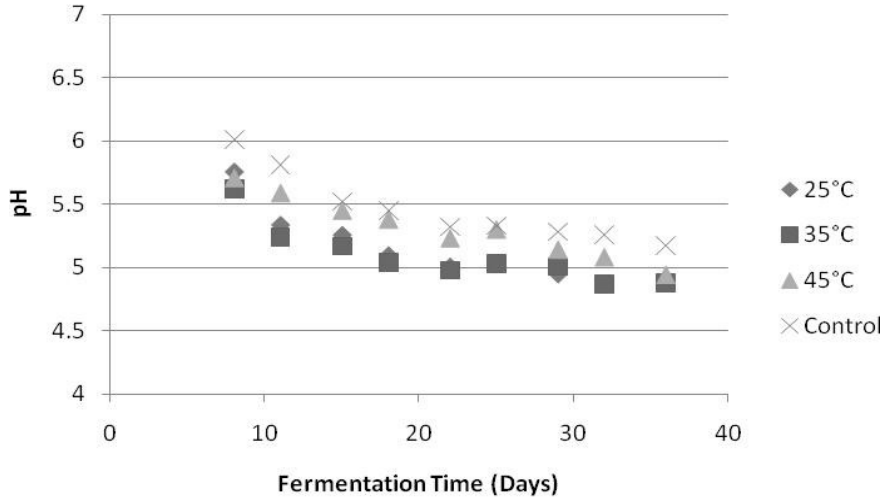

Figure 2. Time courses of pH variations in moromi fermentation.

45℃, while the control was kept under ambient temperature (26-30℃). During this period, the temperature was maintained throughout the process by the jacketed vessel in bioreactor. Aeration was supplied to each bioreactor for 10 min at interval of 3 days, while the aeration was controlled by valve manually.

Initially, the colors of the brine for all the investigated temperatures were green. As the fermentation period increases, the brownish color was formed and the color became darker as the aging of the mashes continued. After one day fermentation, the color of the brine appeared to be the darkest at 45℃ while the color of the brine at 25℃ was still in greenish (Figure 1). However, the color of the brine at 25℃ changed into a darker color after two days of fermentation.

Figure 2 shows the pH variations during the brine fermentation. The pHs of the soy sauce at the fermentation temperature of 25 and 35℃ were lower than the pH of the soy sauce at 45℃ and room temperature. In general, the acidity of the raw soy sauce increased as the fermentation period continued. In the early stage of moromi fermentation, the pH of the soy sauce was about 7.0. As the fermentation period continued, the pH of the soy sauce was reduced to about 4.88 after a month of fermentation. This might be due to the production of lactic acid by *Tetragenococccus halophilus*. Lactic acid was produced through the microbial digestion of the starch that eventually could reduce the pH of the soy sauce (Yong and Wood, 1976). After the pH has dropped below 5.0, *T. halophilus* was unable to grow and an alcoholic fermentation by *Z. rouxii* would begin (Sluis et al., 2001). The decline of pH during fermentation might also be attributed to autolysis of microbial cells, accumulation of free fatty acid, amino acids and peptides containing carbolylic side chains as a result of hydrolysis of materials in soy sauce (Kim and Lee, 2008).

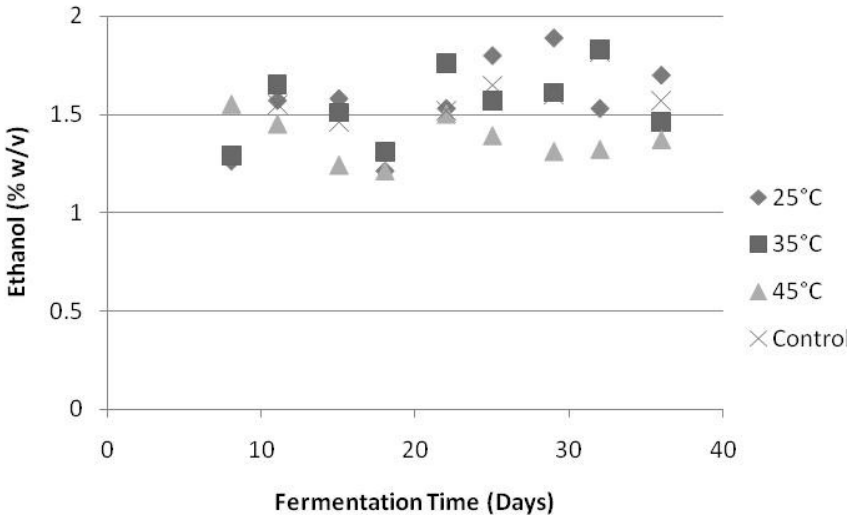

Figure 3. Time courses of ethanol concentrations (% w/v) in moromi fermentation.

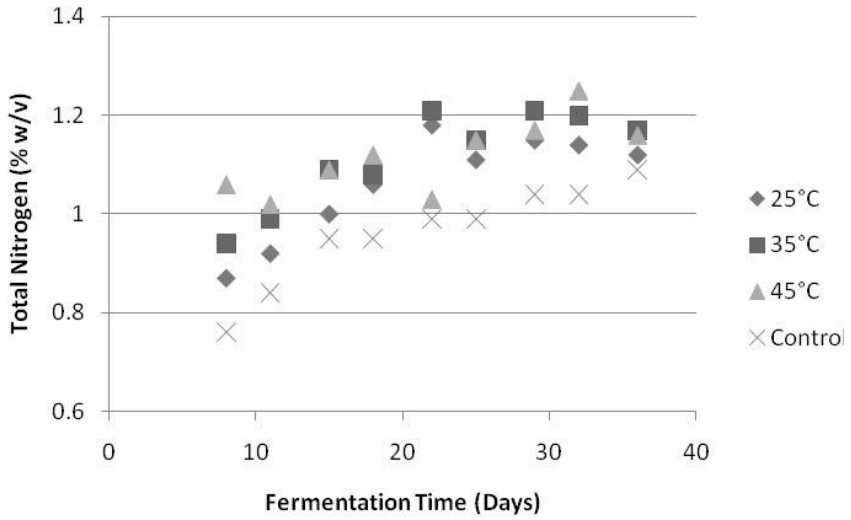

Figure 4. Time courses of total nitrogen contents (% w/v) in moromi fermentation.

Z. rouxii synthesizes ethanol from the sugars that are present during moromi fermentation (Röling, 1995). According to the halal specification in Malaysia, the ethanol content in any halal food must be lower than 2%. Figure 3 shows that the ethanol contents in all trials were below 2%, in which case the lowest content of ethanol was found at the fermentation temperature of 45℃. Seeing that the ethanol concentration is directly proportional to the increasing numbers in yeast cells during the moromi fermentation (Röling et al., 1996), it is not surprising to find that the lowest content of ethanol was found at 45℃ in this experiment because the optimum growth condition of *Z. rouxii* is around 25 - 27.5℃ in brine solution (Hamada et al., 1989; Sasaki and Nunomura, 2003).

Total nitrogen content is an important parameter for evaluating the soy sauce quality (Chou and Ling, 1998). The changes in total nitrogen contents in raw soy sauces at different fermentation temperatures are shown in Figure 4. This study shows that the total nitrogen contents for all soy sauces at different fermentation temperatures were greater than 0.7% (w/v). The total nitrogen contents in all trials were rapidly increasing in the beginning of the fermentation process but their contents were maintained near the end of the experiments. During the fermentation process, the increase of total nitrogen content in the liquid phase might be due to hydrolysis of soy beans. Also, this study shows that the total nitrogen

content in the soy sauce was not greatly influenced by the fermentation temperature (Figure 4). Similar result was also observed by Kim and Lee (2008), in which case they found that the crude protein content in soy sauce was 2.83% at 4°C and only 3.36% (0.53% difference in crude protein content) at 20°C after 360 days of moromi fermentation.

Aeration was introduced in this study because it was considered as one of the important factors in the production of soy sauce. This is because the yeasts generally would not be able to survive during the brine fermentation because of the low availability of oxygen, which is caused by the low aeration rate and poor solubility of oxygen in the brine solution (Sluis et al., 2001). According to Hamada et al. (1989), a supply of air was necessary for vigorous fermentation by *Z. rouxii* as the degree of fermentation was affected by the oxygen transfer rate.

Conclusion

In general, this study indicates that the temperature used in the moromi fermentation imposed significant effects on soy sauce aging and quality. At the highest fermentation temperature of 45°C, the color of the fermentation brine appeared to be the darkest after a certain period of maturation. Also, the lowest content of ethanol in the soy sauce was found at the fermentation temperature of 45°C. On the other hand, the overall pHs of the soy sauce at the fermentation temperature of 25 and 35°C were lower than the pH of the soy sauce at 45°C and room temperature. However, the study found out that the total nitrogen content in the soy sauce was not significantly influenced by the fermentation temperature. Further investigation is required to reconfirm this observation.

ACKNOWLEDGEMENTS

The authors would like to thank the Malaysian Ministry of Science, Technology and Innovation (MOSTI) for supporting this research work under eScienceFund 02-02-10-SF0013. We are grateful to Malaysian Agricultural Research and Development Institute (MARDI) for sponsoring *A. oryzae* and Dr. Yong Wee Ooi for helpful discussions.

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