Miso Fermentation by Mixed Cultures

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Abstract : Miso is a traditional Japanese fermented soybean considered to serve both functions of health benefit and seasonings. Miso provides low cost protein with nutritional values of amino acids and short chain of peptides for consumers. Consumption of this fermented soybean will be an alternative to increase balance of nutritional requirement of protein. The purpose of this study is to examine fermentation characteristics of mixed cultures in miso production. Aspergillus oryzae and Bacillus subtilis were used as mixed cultures in miso fermentation. Results indicated that mixed cultures influenced fermentation characteristics and miso quality. The aerobic plate counts were high (ca.107-1011 CFU g-1) at the onset of miso fermentation but decreased sharply to 105 -109 CFU g-1 in the following 10 days for the miso fermented with mixed cultures. Mixed cultures enhanced acidity but reduced moisture content in the miso products. To apply the mixed cultures in miso production, the appropriate strains and ratios of the cultures should be investigated.

Keywords : yeast cultivation, pineapple juice, molasses, single cell protein

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1. Introduction

Fermented soybean products such as soy sauce or shoyu and miso play a major role in the diet of numerous regions and have been widely consumed by people around the world. It is increasingly recognized that these cultured soy foods are considered to enhance nutritive bioavailability while promoting heart and bone health, and alleviating menopausal symptoms (Minamiyama et al., 2003; Hu et al., 2004; Yamabe et al., 2007; Moktan et al., 2008; Murooka and Yamshita, 2008). Since flavoring components are produced during fermentation of soybean (Beaumont, 2002), umami taste is a key factor that influences these fermented soybean products. Miso is a Japanese traditional fermented soybean considered to serve both functions of health benefit and seasonings. Miso is produced by solid-state fermentation of soybeans, rice or barley with koji mold and then mixed with salt. There are various types of miso depending on proportions of the raw materials (Liu, 1998). Hydrolytic enzymes in koji during fermentation liberates peptides and free amino acids which affects an increase on the nutritive bioavailability (Murooka and Yamshita, 2008). Peptides formed through long term ripening process of miso are considered as taste enhancer (Ogasawara et al., 2006). In addition, miso has antioxidative properties (Giri et al, 2011).

The starter cultures used in the manufacture of miso consist of mold, yeast and bacteria. Well-fermented miso requires the growth of favorable yeasts and lactic acid bacteria, though Aspergillus oryzae or Asperigillus soyae are important mold for koji preparation (Steinkraus, 2004). The osmophilic yeast, e.g. Zygosaccharomyces rouxii and Torulopsis spp. and lactic acid bacteria, e.g. Peiococcus halophilus, Pediococcus cerevisiae and Streptococcus faecalis involve in the moromi fermentation. To control the fermentation, pure culture strains are typically used for inoculum or starter preparation. The inoculation strategies of starter cultures can be separated into pure strain inoculation, where only a single microbial strain is added to substrate and mixed culture inoculation, where the two or more microbial strains are added to substrate (Grossmann et al., 1996; Chomsri, 2008; Gobbi et al., 2013). Mixed culture inoculation could be divided into simultaneous inoculation and sequential inoculation. In the present study, we separately prepared microbial cultures of Aspergillus orzyae and Bacillus subtilis and used them for co-fermentation.

2. Materials and Methods

Microorganism strains and media

Aspergillus oryzae were obtained from the collection of Agricultural Technology Research Institue, Rajamangala University of Technology (RMUTL). The mold was grown at 35 oC in PDA medium PDA (Merck, Darmstadt, Germany). Bacillus subtilis MR 10 came from the collection of Faculty of Science, Maejo University, Thailand. The bacterium was grown at 37 oC on nutrient agar medium; beef extract, 5.0 g/l; peptone, 5.0 g/l; NaCl 2.5 g/l and agar, 15.0 g/l.

Miso fermentation

Soybean, Rajamangala 1 variety, was obtained from Agricultural Technology Research Institute, harvested in 2012. Soybean were selected and cleaned prior to further use. The fermentation process is outlined in Figure 1. A. oryzae was used to prepare koji. The miso was produced by using koji mixed with different proportions of soybean fermented with B. subtilis, i.e. 0, 25 and 100%. The miso was fermented and aged for 60 days by natural fermentation.

Enumeration of microorganisms during miso fermentation

Appropriate serial dilutions of miso samples in saline solution were used for microbiological enumerations. All enumerations were carried out using a plate counting technique. Diluted samples in the saline solution were spread onto a nutrient agar medium for total plate count. Lactic acid bacteria was estimated by using MRS (Merck, Darmstadt, Germany). Yeast and mold count was enumerated by PDA medium. The numbers of colonies were counted after incubation for 1-5 days at 37 oC.

Chemical analyses

Measurement of pH was done by a pH meter (Model C831, Consourt, Belgium). Total acidity was determined by diluting each 5 g of sample in 50 ml distilled water and then titrating to pH 8.2 using 0.1N NaOH. Titratable acidity was expressed as percent lactic acid. Moisture content was measured by the method of Kirk and Sawyer (1991).

Statistics

A completely randomized design (CRD) was applied in this study. Analysis of variance (ANOVA) was used to compare mean differences of the samples. Mean separation was carried out using least significant difference (LSD) for objectively measured data. Statistical significance was assigned as $p < 0.05$.

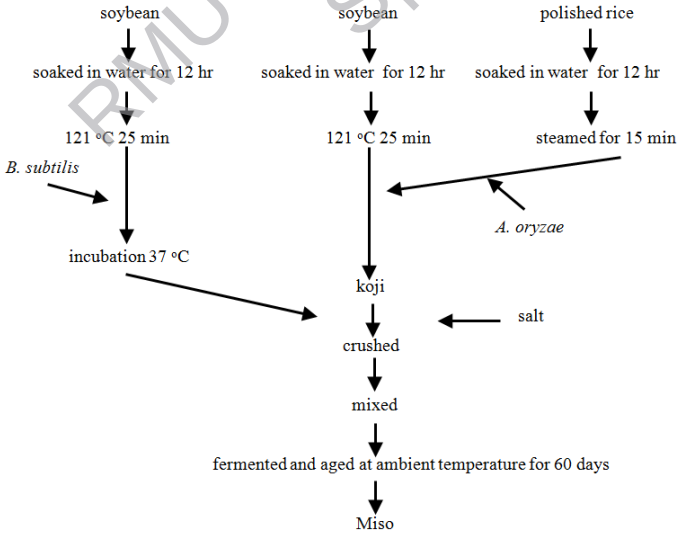

Figure 1. Schematic representation of fermentation of rice-koji miso

3. Results and Discussion

Changes in viable cell counts of microorganisms during miso fermentation

Different proportions of mash prepared by soybean fermented with A. oryzae and B. subtilis were used for miso fermentation and viable cell counts during fermentation were investigated (Figure 2). The aerobic plate counts were ranged between 103 and 1011 CFU g-1. A total plate count reached 1011 CFU g-1. Higher proportion of A. oryzae in the mash showed a little higher of the total plate count. The total viable plate count was fluctuated in 1 log CFU g-1 while Onda et al. (2003) observed that the aerobic bacterial counts were decreased sharply from 106 CFU g-1 to 105 CFU g-1 in the 2nd week of miso fermentation. Lactic acid bacteria count was sharply decreased approximately 1-3 log CFU g-1 after 10 days and consistent until the end of fermentation. Mash containing greater amounts of soybean fermented with B. subtilis had lower yeast and mold counts. Chiou (1999) and Chiou et al. (1999) reported that mold, yeast and LAB populations during miso fermentation were between 3.3-7.6, 5.5-6.3 and 4.5-6.7 log CFU g-1 respectively. Based on mixed cultures in miso fermentation at mashing step, the results suggested there was the influence of microbial interaction on growth of microorganisms during miso fermentation.

Changes in pH, total acidity and moisture content during miso fermentation

Changes in pH, total acidity and moisture content of miso fermented with mixed cultures during fermentation are presented in Figure 3. pH values and total acidity during miso fermentation were not significantly affected by mashes prepared by different proportions of soybean fermented with A. oryzae and B. subtilis. During fermentation pH was decreased which was corresponded to an increase of total acidity. Miso fermented without addition of soybean fermented with B. subtilis was slightly decreased of total acidity after 30 day of fermentation while total acidity of soybean fermented with mixed cultures was slightly increased. Considering pH values, miso fermented with different ratios of soybean prepared from A. oryzae and B. subtilis showed very similar profiles. This may be described by hydrolytic enzymes produced diversity of substances such as amino acids, peptides and phenolic compounds which could affect buffer property of miso (Ogasawara et al., 2006; Champagne et al., 2009; Giri et al., 2011). Moisture content was gradually reduced from 51.00-54.55% at the onset of fermentation to 45.25-50.91% in the following 60 days. Lower moisture contents were observed in mash prepared by addition of soybean fermented with B. subtilis.

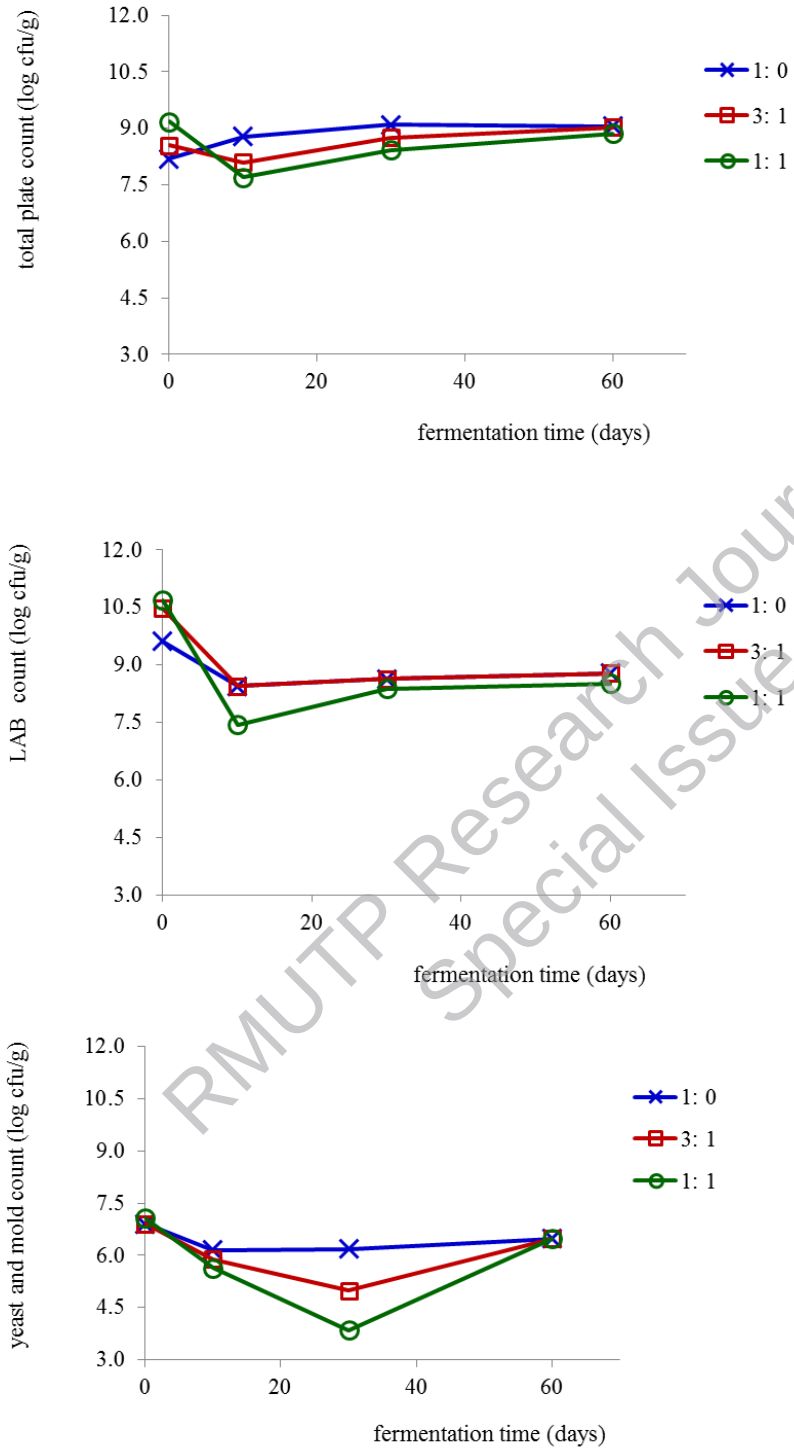

Figure 2 Changes in total viable count (a), lactic acid bacteria (b) and yeast and mold during miso fermentation process; different ratios of soybean fermented with A. oryzae and B. subtilis were used to produce miso, i.e. 1:0, 3:1 and 1:1

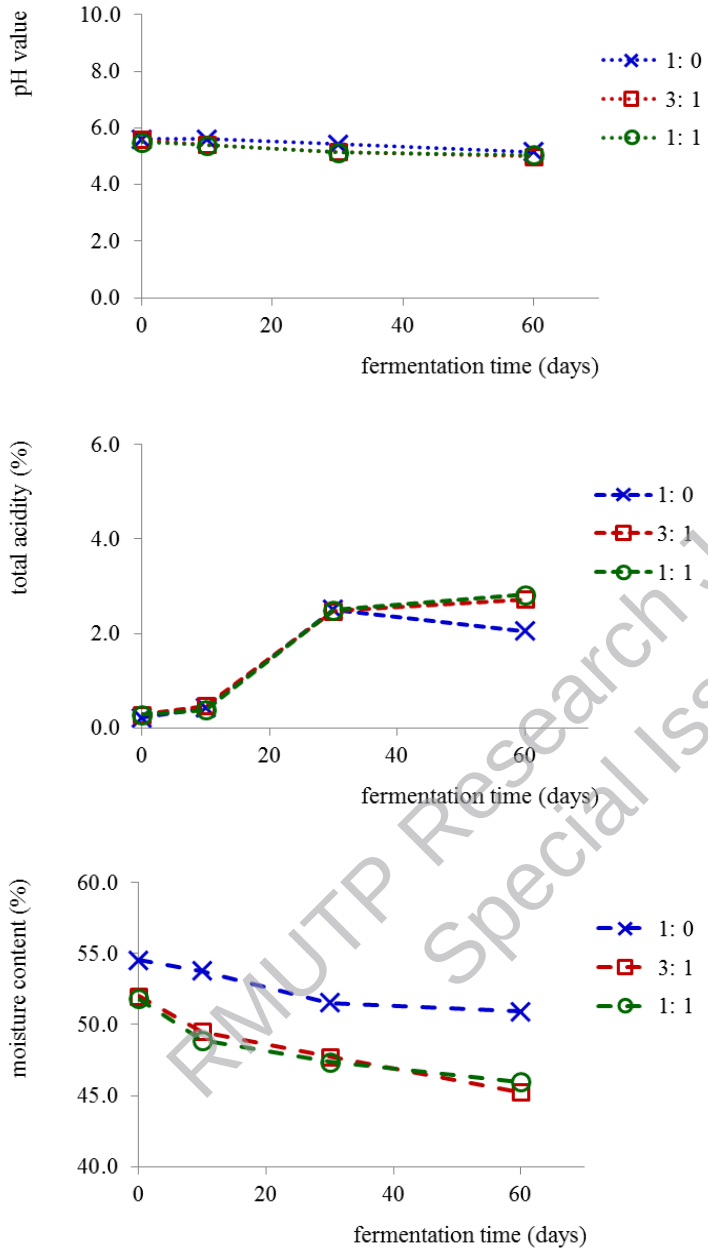

Figure 3 Changes of pH (a), total acidity (b) and moisture content (c) during miso fermentation process; different ratios of soybean fermented with A. oryzae and B. subtilis were used to produce miso, i.e. 1:0, 3:1 and 1:1

Chemical properties of miso products

Chemical properties of miso products prepared from mash with different proportion of soybean fermented with A. oryzae and B. subtilis shows in Table 1. Shukla et al. (2011) reported that pH of miso was ranged from 4.8 to 5.5. The pH values of three miso products in this study were 4.99-5.15 (Table 1) which is in agreement with previous report. The miso products showed total acidity of 2.04-2.83%. Lactic acid produced from bacteria and amino acid possessing acidic property may be responsible for this high quantity of total acidity. The moisture contents of the miso products in this study (45.25-50.91%) were significantly different ($p<0.05$). This data support the literature with respect to the moisture contents in miso ranged between 30.6-53.0 % (Liu, 1997; Chiou, 2001). The use of koji prepared from cooked soybean fermented with A. oryzae for miso production gained higher moisture content in the final product in comparison to mash prepared from mixed proportions of A. oryzae and B. subtilis. This could be an evidence of the effect of mixed cultures on miso fermentation.

Table 1 chemical property of miso products prepared by koji mixed with different proportions of soybean fermented with B. subtilis

treatment†	pH	total acidity (%)	moisture content (%)*
1:0	5.15+0.14ns	2.04+0.70 ns	50.91+1.31a
3:1	4.99+0.14 ns	2.53+0.52 ns	45.25+4.09b
1:1	5.03+0.28 ns	2.82+0.26 ns	45.96+0.19b

† ratios of soybean fermented with A. oryzae and B. subtilis for miso production
* Means in a column with the different letters represent significant differences ($p<0.05$)
ns denotes means are not significantly different ($p>0.05$)

4. Conclusion

The use of mixed culture strategy for miso production affected the microbiological and chemical changes during fermentation process. An increase of Bacillus subtilis proportion applied in miso production process in this study had an impact on reduction of yeast and mold counts during fermentation. The use of mixed cultures, i.e. Aspergillus oryzae and B. subtilis for miso fermentation decreased moisture content of miso product. The results provide valid evidence that mixed cultures impact miso quality. Further investigation in this area should be carried out. The study on different protocols of mixed cultures such as a use of different strains or a use of yeast in mixed culture and sensory characters.

5. Acknowledgements

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