Abstract

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(5th RMUTIC)

"Technology and Innovation towards ASEAN"

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Information Technology Building

Rajamangala University of Technology Suvarnabhumi

Phranakhon Si Ayutthaya, Thailand

03.35 – 03.55 p.m.	5ES-O17	English Collaborative Teaching for Students with Low Level of English Communication Behaviors	Pornchai Pornwiriyakit / RMUTTO
03.55 – 04.15 p.m.	5ES-O18	The Study on Reading Comprehension Utilizing Intensive Reading of Simplified Stories, St Theresa International College	Yongyut Khamkhong / STIC
04.15 – 04.35 p.m.	5ES-O19	Proposing development of strategy for sustainable tourism of Patong Beach, Phuket Island, Thailand	Maythawin Polnyotee / CU
04.35 – 04.55 p.m.	5ES-O20	Utilization of Informaiton Technology on Interner by Undergraduate students	Pawana Poomsawai / RMUTSV

Session 8 Agricultural and Food Industry **Room: 240302**

Chairman	Assoc. Prof. Udomluck Muchjajib		RMUTSB
Co-chair	Asst. Prof. Dr. Naiyawit Chalermnon		RMUTSB
08.30 – 08.50 a.m.	5AF-O01	Miso Produced from Different Thai Rice Cultivars : Physicochemical and Sensory Characteristics	Supojjanee Intaramoree / RMUTL
08.50 – 09.10 a.m.	5AF-O02	Downy Mildew Resistant and Susceptible Cucumber Inbred Lines	Chanulak Khanobdee / RMUTL
09.10 – 09.30 a.m.	5AF-O03	Consumer Study of Mulberry Wine Fermented by *Saccharomyces* Yeast Co-Inoculation	Ni-orn Chomsri / RMUTL
09.30 – 09.50 a.m.	5AF-O04	Stability Analysis of Six Super Sweet Corn Cultivars under Chemical and Organic Fertilizer Growing Systems	Pramote Pornsuriya / RMUTTO
09.50 – 10.10 a.m.	5AF-O05	Chemical and Microbial Changes during the Production of Fish Sauce from Pacific White Shrimp (*Litopenaeus vannamei*) Processing By-Products	Yardrung Suwannarat / RBRU
10.10 – 10.25 a.m.	**Coffee Break**		
Chairman	Prof. Dr. Henry Ho-Hsien Chen		NPUST
Co-chair	Dr. Wijitra Liaotrakoon		RMUTSB
10.25 – 10.45 a.m.	5AF-O06	Effects of Hydrothermal Treatment on Rheological Properties and Texture of High-Amylose Rice Flour	Supawadee Cham / RMUTTO
10.45 – 11.05 a.m.	5AF-O07	Efficiency of Moisture Absorber and Vacuum Packing Using on Microbiological Quality of Khanom Jaak	Boondarika Sumana / RMUTTO
11.05 – 11.35 a.m.	5AF-O08	Actinomycetes metabolite and plant elicitors affecting to chili plant defense related genes	Nisakorn Suwan / RMUTTO
11.35 – 11.55 p.m.	5AF-O09	The use of Tempering and Pricking to Improve Quality of Cassava Drying	Phanida Busaparoek / RMUTT
12.00 – 01.00 p.m.	**Lunch Break**		

5AF-O01: Miso Produced from Different Thai Rice Cultivars: Physicochemical and Sensory Characteristics

Supojjanee Intaramoree[1] and Ni-orn Chomsri[1*]

Abstract: Miso, a Japanese traditional fermented soybean product, exists in three forms, rice miso, barley miso and soybean miso. This work evaluated rice miso prepared from two Thai rice cultivars, Hommali and Homnil by examining physicochemical and sensory properties. After a 2-week fermentation the results showed that the rice cultivars themselves had a significant effect on miso color values of lightness (L*), redness (a*) and yellowness (b*). The miso products contained 20.81-32.70 mg/g of protein, 247-466 µmole/g of free alpha amino nitrogen and 164.57-281.55 mg/g of reducing sugars. When samples were tested for ABTS radical-scavenging activity, it was found that miso prepared by Homnil rice cultivar exhibited higher scavenging activity than miso prepared by Hommali rice cultivar. Sensory analysis of miso soups showed that appearance, color, umami and overall acceptance attributes in the miso soups were influenced by the rice cultivars used in miso fermentation. The present study implies that miso fermented using different Thai rice cultivars can provide different qualities for the consumers, and have the potential to be developed into nutritious and functional products.

Keywords: miso, rice, fermentation

[1] Department of Research and Development, Agricultural Technology Research Institute, Rajamangala University of Technology Lanna, Lampang, 52000

[*] Corresponding author. Email: niornchomsri@rmutl.ac.th, niornchomsri@yahoo.de

Miso Produced from Different Thai Rice Cultivars: Physicochemical and Sensory Characteristics

Supojjanee Intaramoree and Ni-orn Chomsri[1]*

Abstract

Miso, a Japanese traditional fermented soybean product, exists in three forms, rice miso, barley miso and soybean miso. This work evaluated rice miso prepared from two Thai rice cultivars, Hommali and Homnil by examining physicochemical and sensory properties. After a 2-week fermentation the results showed that the rice cultivars themselves had a significant effect on miso color values of lightness (L*), redness (a*) and yellowness (b*). The miso products contained 20.81-32.70 mg/g of protein, 247-466 μmole/g of free alpha amino nitrogen and 164.57-281.55 mg/g of reducing sugars. When samples were tested for ABTS radical-scavenging activity, it was found that miso prepared by Homnil rice cultivar exhibited higher scavenging activity than miso prepared by Hommali rice cultivar. Sensory analysis of miso soups showed that appearance, color, umami and overall acceptance attributes in the miso soups were influenced by the rice cultivars used in miso fermentation. The present study implied that miso fermented using different Thai rice cultivars could provide different quality attributes for the consumers, and had the potential to be developed into nutritious and functional products.
Keywords: miso, rice, fermentation

[1] Department of Research and Development, Agricultural Technology Research Institute, Rajamangala University of Technology Lanna, Lampang 52000, Thailand

*Corresponding author. Email: supojjanee_intaramoree@hotmail.co.th

INTRODUCTION

Soybean is an excellent source for nutrition that include plant protein, oligosaccharides, vitamin B, vitamin E and mineral substances with some functional compounds which can reduce the risk of cardiovascular diseases and cancers (Zhang et al., 2014). Fermentation of soybean can improve the nutritional utilization of soybean because microbial enzyme degrades insoluble macromolecular substances; such as protein, fat and carbohydrate into polypeptides, fatty acids, oligopeptides and amino acids (Murooka and Yamshita, 2008; Giri et al., 2011; Chancharoonpong et al., 2012; Zhang et al., 2014). Amino acids, i.e. liberated glutamate and peptides during fermentation are taste enhancers in fermented products, e.g. miso, soy sauce. Miso is a Japanese traditional fermented soybean considered to serve both functions of health benefit and seasonings with consumption dating back to A.D. 700 (Yoshinaga et al., 2012; Marui et al., 2013). Miso is a sort of two-stage fermentation process. The first stage involves the production of the koji from cooked grains inoculated with *Aspergillus oryzae* and a second fermentation (the moromi) is the koji mixed with salt, cooked soybeans, and appropriate inoculums (Abiose et al., 1982). Miso provides low cost protein with nutritional values of amino acids and short chain of peptides for consumers. Although, there are various types of miso, they can be classified into three major types on the basis of raw materials employed, e.g. rice (kome) miso, barley (mugi) miso and soybean (mame) miso (Steinkraus, 2004; Kikkoman Corporation, 2014).

Thailand, a major rice production of the world and is the largest rice exporter in the world with the total volume of about 5-6 million tons per year. Many rice cultivars have been grown and available in the market. Regarding to the different quality of rice cultivars (Naivikul, 2013), it would be a useful information to study the effect of different rice cultivars on miso quality. In this research, the solid-state fermentation of whole soybean mixed with Thai rice was performed. Physicochemical and sensory characteristics were examined in miso products.

MATERIALS AND METHODS

Microorganism and starter preparation

Aspergillus oryzae was obtained from the collection of Agricultural Technology Research Institue, Rajamangala University of Technology (RMUTL). The mold was grown at 35 °C in PDA medium PDA (Merck, Darmstadt, Germany). The starter was prepared by inoculating the mold on rice which was sterilized at 15 lb/in^2 for 15 min. The inoculated rice was then incubated at 35 °C for 5 days. The rice overgrown with mold was then used as a starter for koji production.

Koji fermentation

Soybean, Rajamangala 1 variety, was obtained from Agricultural Technology Research Institute, harvested in 2013. Soybean seeds were selected and cleaned prior to further use. Whole soybean was washed and then soaked in water that was five times of the soybeans weight at ambient temperature overnight. After water draining, soybean was washed, cooked in an autoclave (121 °C, 20 min) and cooled. Solid state fermentation was performed by addition of 1% starter into the cooked soybean substrate. After mixing thoroughly, the inoculated soybean substrate was placed on a bamboo tray and incubated for 30 h at ambient temperature.

Miso fermentation

Rice cultivar and ratio of soybean and rice were two factors in this study. Miso fermentation by two cultivars of rice, i.e. Hommali and Homnil and two ratios of soybean and rice, i.e. 1:1 and 2:1 was carried out. The brewing process was performed as described in Chomsri et al. (2013). The ground koji was mixed with cooked and ground rice. The mixture was placed in glass jar and aged for two weeks at ambient temperature. Two replicate experiments was conducted.

Physicochemical analyses

Color was determined with a color meter (JS555, Juki, Japan). Measurement of pH was carried out by a pH meter (Model C831, Consourt, Belgium). Absorbency at 450 nm (A_{450}) was employed for measurement of color change in water-soluble fraction of miso (Ogasawara et al., 2006). 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). Ninhidrin colorimetric method was used to measure free alpha amino nitrogen (FAN) in the juice and the fermented juice (Wylie and Johnson, 1961). Antioxidant activity assay was determined by using the ABTS methods as described by Wongputtisin et al. (2007). Reducing sugars were determined with dinitrosalicylic acid (DNS) method (Miller, 1972).

Sensory evaluation

All the panelists were experienced in fermented soybean products. A group of 30 panelists took part in this study. Miso products fermented for 2 weeks were evaluated for organoleptic quality. Boiling water was poured to 10 g of miso, mixed and directly served to panels. The panelists were asked to rate the products on the 9-point hedonic scale (Meilgaard *et al*., 1991).

Statistics

The experimental designs of 2x2 factorial in CRD (completely randomized design) for physicochemical analysis and in RCBD (randomized complete block design) for sensory analysis were applied in this study. Analysis of variance (ANOVA) was used to compare mean differences of the samples. Mean separation was carried out using DMNRT (Duncan's new Multiple Range Test) for objectively measured data. Statistical significance was assigned as $p \leq 0.05$.

RESULTS AND DISCUSSION

Table 1 showed the color values of miso fermented two weeks at different rice cultivars and ratios. The fermented misos showed L*, a* and b* values of 27.86 to 43.59, 8.20 to 11.44 and 13.84 to 24.54, respectively. Different soybean and rice ratios of 1:1 and 2:1 were not found to affect the lightness (L*) of the misos after fermentation for two weeks ($p>0.05$), while the use of Homnil rice cultivar in miso fermentation produced darker color than the use of Hommali rice cultivar ($p\leq 0.05$). The a* and b* values indicated red-green and yellow-blue colors, respectively. The results showed that miso made from Hommali rice cultivar and higher soybean content had higher red color (a*) and blue color (b*) than miso made from Homnil rice cultivar and lower soybean content ($p\leq 0.05$). This demonstrated that the rice the rice cultivars and their amounts in the misos influenced color components in the final products, however, components generated during fermentation might also have an impact on miso color quality Chiou et al., 1999; Ogasawara et al., 2006).

Table 1 Color values of misos made by different rice cultivars and ratios after two-week fermentation.

Factor	L*	a*	b*
factor A (rice cultivar)			
A_1 : Hommali	39.76 ± 4.60^{a}	10.16 ± 1.50^{a}	24.36 ± 0.76^{a}
A_2: Homnil	28.71 ± 1.26^{b}	8.95 ± 0.86^{b}	16.02 ± 2.59^{b}
factor B (soybean and rice ratio)			
B_1: 1:1	35.72 ± 9.20^{ns}	8.54 ± 0.41^{b}	19.19 ± 6.22^{b}
B_2: 2:1	32.24 ± 3.68^{ns}	$10.57\pm 1..12^{a}$	21.19 ± 3.49^{a}
Interaction			
$A_1\times B_1$	43.59 ± 2.26^{a}	8.88 ± 0.19^{ns}	24.54 ± 0.95^{a}
$A_1\times B_2$	35.93 ± 0.12^{b}	11.44 ± 0.85^{ns}	24.18 ± 0.84^{a}
$A_2\times B_1$	27.86 ± 1.26^{c}	8.20 ± 0.08^{ns}	13.84 ± 0.98^{c}
$A_2\times B_2$	29.57 ± 0.55^{c}	9.70 ± 0.13^{ns}	18.20 ± 0.43^{b}

Within a column different letters denote significant differences ($p\leq 0.05$)
ns denotes means are not significantly different ($p>0.05$)

The color of the miso became browner during fermentation as indicated by A_{450} (Figure 1). The absorbance of the water-soluble fraction increased ca. 1-3 times after 2 weeks of fermentation. Darker color of the water-soluble fraction of miso was also observed in miso ripening for 20 months due to Maillard reaction (Ogasawara et al., 2006). However, rice cultivars affected the color of water-soluble fractions of miso. The

Table 2 Protein and free alpha amino nitrogen (FAN) contents of misos made by different rice cultivars and ratios.

Factor	Protein content (mg/g)		FAN content (μmol/g)	
	0 D	2 weeks	0 D	2 weeks
factor A (rice cultivar)				
A_1 : Hommali	29.46±4.03[ns]	26.23±2.33[a]	23.21±2.24[b]	356.58±128.54[ns]
A_2: Homnil	35.97±4.13[ns]	24.20±2.49[b]	36.34±10.45[a]	358.54±116.13[ns]
factor B (soybean and rice ratio)				
B_1: 1:1	30.62±5.18[ns]	26.69±51.20[a]	33.49±13.78[a]	351.07±124.69[ns]
B_2: 2:1	34.80±4.80[ns]	23.74±2.67[b]	26.06±1.59[b]	364.33±119.78[ns]
Interaction				
$A_1 \times B_1$	27.54±5.81[ns]	27.15±1.57[ns]	21.62±1.90[c]	246.94±18.24[b]
$A_1 \times B_2$	31.38±0.62[ns]	25.30±3.23 [ns]	24.80±1.15[bc]	466.76±30.27[a]
$A_2 \times B_1$	33.71±2.96[ns]	26.22±0.99 [ns]	45.37±1.42[a]	455.20±54.24.[a]
$A_2 \times B_2$	38.23±4.69[ns]	22.19±1.15 [ns]	27.31±0.16[b]	261.89±12.41[b]

Within a column different letters denote significant differences ($p \leq 0.05$)
ns denotes means are not significantly different ($p > 0.05$)

Determination of reducing sugars revealed that rice cultivars influenced the reducing sugar contents in miso products (Table 3). Miso fermented by addition of Homnil rice had higher reducing sugar content than Hommali rice. The structure of starch in the Homnil rice could be a reason because it might be easily digested by the mold amylase applied in this study (Naivikul, 2013). The extract of miso after two-week fermentation was assayed for ABTS radical scavenging capacity. The results suggested that miso fermented by addition of Homnil rice produced higher scavenging activity of 76.45% than addition of Hommali (51.69%). With regard to the soybean and rice ratio, it was found that higher content of soybean significantly enhanced higher scavenging activity ($p \leq 0.05$).

Table 3 Antioxidant activity and reducing sugars content of misos made by different rice cultivars and ratios after two-week fermentation.

Factor	Reducing sugar content (mg/g)	Scavenging capacity (%)
factor A (rice cultivar)		
A_1 : Hommali	179.94 ± 19.20^{b}	61.69 ± 17.80^{b}
A_2: Homnil	261.38 ± 28.19^{a}	76.45 ± 2.18^{a}
factor B (soybean and rice ratio)		
B_1: 1:1	223.06 ± 68.13^{ns}	61.46 ± 17.64^{b}
B_2: 2:1	218.26 ± 30.45^{ns}	76.68 ± 1.07^{a}
Interaction		
$A_1 \times B_1$	164.57 ± 12.30^{b}	46.30 ± 0.90^{b}
$A_1 \times B_2$	195.32 ± 3.05^{b}	77.09 ± 1.35^{a}
$A_2 \times B_1$	281.55 ± 9.48^{a}	76.63 ± 3.63^{a}
$A_2 \times B_2$	241.21 ± 25.82^{a}	76.28 ± 0.98^{a}

Within a column different letters denote significant differences ($p \leq 0.05$)
ns denotes means are not significantly different ($p>0.05$)

Sensory scores of 2-week fermented miso products were shown in Table 4 Rice cultivars had an impact on sensory quality of miso products. The results indicated that using Hommali rice cultivar for miso fermentation yielded the products with significantly higher sensory scores of appearance, color, umami taste and overall preference attributes ($p \leq 0.05$). The effect of soybean and rice ratio on sensory quality of the miso products was not observed. The four misos recieved scores at the level of "like slightly" to "like very much" for the quality attributes of appearance, color, odor, umami taste and overall preference ($p>0.05$).

Table 4 Mean scores for sensory attributes of miso soups prepared from two-week fermented misos with different rice cultivars and ratios after two-week fermentation.

Factor	appearance	color	odor	umami	overall
factor A (rice cultivar)					
A_1 : Hommali	7.30±0.99[a]	7.21±1.10[a]	6.63±1.09[ns]	6.81±1.21[a]	6.97±1.15[a]
A_2: Homnil	6.30±1.17[b]	6.05±1.19[b]	6.32±1.22[ns]	6.21± 1.40[b]	6.26±1.31[b]
factor B (soybean and rice ratio)					
B_1: 1:1	6.76±1.09[ns]	6.53±1.25[ns]	6.46±1.08[ns]	6.56±1.31[ns]	6.61±1.35[ns]
B_2: 2:1	6.83±1.29[ns]	6.72±1.32[ns]	6.50±1.26[ns]	6.46±1.37[ns]	6.62±1.22[ns]
Interaction					
$A_1 \times B_1$	7.15±0.87[ns]	6.97±1.03[ns]	6.62±1.03[ns]	6.87±1.12[ns]	7.07±0.97[ns]
$A_1 \times B_2$	7.45±1.09[ns]	7.45±1.14[ns]	6.65±1.18[ns]	6.75±1.33[ns]	6.87±1.33[ns]
$A_2 \times B_1$	6.37±1.17[ns]	6.10±1.33[ns]	6.30±1.12[ns]	6.25±1.44[ns]	6.15±1.53[ns]
$A_2 \times B_2$	6.22±1.19[ns]	6.00±1.07[ns]	6.35±1.34[ns]	6.17±1.38[ns]	6.37±1.08[ns]

Within a column different letters denote significant differences ($p \leq 0.05$)
ns denotes means are not significantly different ($p > 0.05$)

CONCLUSION

There are many types of miso depending on ingredient utilization and fermentation condition. This study provided the results of different cultivars of rice and different ratios of soybean and rice had an impact on physicochemical and sensory quality of miso products. The obtained data can support miso production in Thailand as the Thai rice was used in this research. However, various aspects of investigation such as other potential rice cultivars, different amounts of ingredients, microbial change during fermentation, shelf-life study and consumer test, are needed to be explored

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REFERENCES

Abiose, S.H., Allan, M.C. and Wood, B.J.B. 1982. Microbiology and biochemistry of miso (soy paste) fermentation. Advances in Applied Microbiology 28: 239–265.

Chiou, R.Y.Y., Ferng, S. and Beuchat, L.R. 1999. Fermentation of low-salt miso as affected by supplementation with ethanol. International Journal of Food Microbiology 48: 11–20.

Chomsri, N., Intaramoree, S. and Sunantha, W. 2013. Miso Fermentation by Mixed Cultures RMUTP Research Journal Special Issue International Conference: 249-257.

Chomsri, N., Pongjunta, J., Nualbunruang, P. and Intaramoree, S. 2013. Utilization of broken rice starch in a single-step liquefaction and saccharification process. Food Innovation Asia Conference 2013: Empowering SMEs through Science and Technology, 13-14 June 2013, BITEC, Bangkok, Thailand.

Giri, A., Osako, K., Okamoto, A., Okazaki, E. and Ohshima, T. 2011. Antioxidative properties of aqueous and aroma extracts of squid miso prepared with *Aspergillus oryzae*-inoculated koji. Food Research International 44: 317-325.

Kikkoman Corporation, 2014. Fermented Foods of Japan: Miso. (online). Available: http://www.kikkoman.com/foodforum/thejapanesetable/26.shtml (25 Jun. 2014).

Kirk, R and Sawyer, R. 1991. Pearson's compostion and analysis of foods. Longman Scientific and Technical. Essex. 708 p.

Marui, J., Tada, S., Fukuoka, Mari., Wagub, Y., Shiraishi, Y., Kitamotoc, N., Sugimoto, T., Hattori, R., Suzuki, S. and Kusumoto, K. 2013. Reduction of the degradation activity of umami-enhancing purinic ribonucleotide supplement in miso by the targeted suppression of acid phosphatases in the *Aspergillus oryzae* starter culture. International Journal of Food Microbiology 166: 238–243.

Meilgaard, H., Civille, G.V. and Carr, B.T. 1991. Sensory Evaluation Techniques. CRC Press, Boca Raton. 354 p.

Miller, G.L. 1972. Use of dinitrosalicylic acid reagent for determination of reducing sugars. Analytical Chemistry 31: 426-428.

Ogasawara, M., Yamada, Y. and Egi, M. 2006. Taste enhancer form the long-term ripening of miso (soybean paste). Food Chemistry 99: 736-741.

Royal Thai Embassy Oslo, Norway. 2013. Thailand deposit 81 varieties of rice seed in the Svalbard Global Seed Vault. (online). Available: http://www.thaiembassy.no/wordpress/blog/thailand-deposit-81-varieties-of-rice-seed-in-the-svalbard-global-seed-vault/. (25 Jun. 2014).

Steinkraus, K.H. 2004. Industrialization of indigenous fermented foods. Marcel Dekker, Inc. New York. 796 p.

Wongputtisin, P., Khanongnuch, C., Pongpiachan, P., and Lumyoung, S. 2007. Antioxidant activity improvement of soybean meal by microbial fermentation. Research Journal of Microbiology 2(7): 577-583.

Wylie, E. B., and Johnson, M. 1961. Effect of penicillin on the cell wall of *Escherichia coli*. Biochimica et Biophysica Acta 59, 450-457.

Yoshinaga, M., Toda, Natsuko., Tamura, Y., Terakado, S., Ueno, Mai., Otsuka, K., Numabe, A., Kawabata,Y., and Uehara, Yoshio. 2012. Japanese traditional miso soup attenuates salt-induced hypertension and its organ damage in Dahl salt-sensitive rats. Nutrition 28: 924–931.

Zhang, S., Shi, Y., Zhang, S., Shang, W., Gao, X. and Wang, H. 2014. Whole soybean as probiotic lactic acid bacteria carrier food in solid-state fermentation. Food Control 41: 1-6.