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# Production of Seasoning "Mirin" from Thai Rice by Fermentation

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## ABSTRACT

The investigation of the use of *Aspergillus oryzae* strain and Thai non-glutinous rice varieties, which are suitable for enzyme production for koji preparation was carried out. *A. oryzae* strain no. WM-2 could produce a-amylase enzyme at the highest level while "Leung 11", a non-glutinous rice variety was the most suitable for koji preparation because of its unaggregation characteristics which are good for enzyme production (31.71 units/ml of a-amylase and 6.66 units/ml of acid protease). The suitable ratio of solid:liquid content for mirin production was also studied and it was found that the ratio of 60:40 was the most appropriate because it gave rather pale color and suitable residual alcohol concentration (13 % v/v). This ratio was subsequently used for the determination of an appropriate ratio of koji to glutinous rice. The result indicated that the ratio of koji to glutinous rice of 1:7 gave good quality mirin when comparing with the commercial mirin. Based on the scaling-up of koji preparation, it was found that the cultivation time of 36 h gave the highest activities of a–amylase and protease, which are suitable for mirin production. Ten kilograms of rice koji with 1.0 inch bed thickness gave suitable conditions for enzyme production (322.0 units/g dry wt. of a–amylase and 150.82 units/g dry wt. of acid protease). The results from the study were used for pilot-scale mirin production (50 kg). Ninety per cent of the untrained panelists accepted the quality of mirin produced at the above-mentioned conditions.


**Key words:** amylase, mirin, rice, seasoning, solid state fermentation

## INTRODUCTION

Mirin is a traditional alcoholic seasoning, which has been used widely in Japan. It is manufactured from steamed glutinous rice, koji and brewing alcohol. Mirin improves flavor and viscosity of food products and is used in many food industries and Japanese restaurants. Thailand has been a continuous importer of mirin although the country is one of the most important rice producers in the world. It is therefore interesting to study the production of mirin from thai rice in order to reduce the import of mirin and also to add value to abundantly available rice in Thailand. In general, commercial mirin compositions consist of approximately 38-40% reducing sugar, 0.06-0.08% nitrogen, 10-13 %(v/v) alcohol and pH 5.8-5.9 (Uchida and Oka, 1983)

The purposes of this study were to investigate a suitable strain of *Aspergillus oryzae* and a variety of non-glutinous rice for koji preparation. The determination of appropriate ratios of solid to liquid content as well as of obtained koji to glutinous rice to produce mirin, which has comparable quality to that of the commercial one was also performed.

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## MATERIALS AND METHODS

### Materials

Seven varieties of non-glutinous rice, namely, Supanburi 1, RD19, RD23, Khao Dawk Mali 105, Leung 11, Chai Nat 1 and Leung-aun were supplied by Bangkhen Rice Research Institute and Kalasin Rung-Reung no. 3 Rice Mill. The glutinous rice variety RD6 was also supplied by the same institution.

Twelve strains of *Aspergillus oryzae* were isolated from Japanese commercial spore powder and culture collection at the Department of Biotechnology's laboratory, Faculty of Agro-Industry. These fungi were cultured in potato dextrose agar (PDA) at 30∂C for 7 days.

### Tane koji preparation

Fifty milligrams of non-glutinous rice was washed, soaked overnight at room temperature (30±2∂C) and drained. The sample was then transferred to a 250 mL flask. Rice was steamed in an autoclave under pressure at 121 ∂C for 30 min. After cooling down, fungal inoculum was inoculated into rice, mixed and incubated at 30 ∂ C for 7 days.

### Study on fungal strains and screening of non-glutinous rice varieties for preparation of koji

One kilogram of non-glutinous rice (Leung-aun variety) was washed and soaked in water for 3 h at room temperature (25-34∂C depending on the season). After the excess water was drained, the soaked rice was wrapped in muslin clothes and steamed for 30 min. 1-5% (w/w) tane koji was then mixed with steamed rice, which was first cooled down to 42∂C. The mixture was incubated at 30∂C for 40-46 h. The fungal strain, which gave the highest enzyme activity from 12 tested strains, was chosen to prepare koji using 7 varieties of non-glutinous rice. Extracted enzymes from koji were assayed for a-amylase activity using starch-iodine complex method and protease activity using 1.2% (w/v) casein (Kanlayakrit, 1987) as the substrate.

### Laboratory-scale mirin production

Glutinous rice (variety RD6) was soaked in water for 3 h at room temperature. The water was then discarded and soaked rice was steamed for 40 min. The mash (2 kg) was composed of steamed glutinous rice mixed with koji and 35% (w/w) ethanol. To identify the optimum ratio of raw materials for mirin-making, the mash (2 kg) was prepared with the ratio of koji:glutinous rice of 1:6.75 and various ratios of solid:liquid (koji and glutinous rice/35% alcohol: 72.1/27.9, 70/30, 60/40 and 50/50). Fermentation was carried out for 45-60 days at room temperature (25-34∂C depending on the season). Mirin characteristics from various fermentation ratios (koji, steamed glutinous rice and volume of ethanol) were then compared.

### Pilot-scale production of koji

Steamed non-glutinous rice (5, 10 and 15 kg) was inoculated with tane koji (10%) for koji preparation. After transferring and spreading inoculated rice into a koji machine, rice bed thickness were adjusted to 0.5, 1.0 and 2.0 inch. Humid air at 30 ∂C was introduced for 60 h. Fungal growth and enzyme productivity were compared with those of koji obtained from the laboratory-scale production. Koji from this experiment was subsequently used for pilot-scale production of mirin.

### Pilot-scale production of mirin

In a 100 liters plastic bucket, the mash (50 kg) was prepared with the chosen formula from the above-mentioned experiments. The mixture was inoculated for 60 days. The mash was then pressed manually through thin white cloth to obtain mirin. Mirin quality was then compared with that of commercial mirin using 9-point hedonic sensory evaluation scale.

**Analytical methods**

Acid analysis was performed according to the Amerine method (1979). pH and alcohol analysis were performed by the dichromate oxidation method (Amerine and Ough, 1974). Total soluble solid was measured using a refractometer. Reducing sugar was determined by the DNS method (Miller, 1959). Color and clearness was measured using spectrophotometer at 440 and 660 nm, respectively. Glucosamine was determined by the Blix method (1948).

## RESULTS AND DISCUSSION

By screening for fungal strains producing the highest enzyme activities, it was found that strains no. WM-1 and WM-2 showed the highest acid-protease and a-amylase activities (8.94 and 28.7 units/ml, respectively) (Fig 1). As almost all of raw materials for mirin production was starch, a–amylase enzyme played a more important role than did acid-protease in the production of mirin. This criteria was used to select the appropriate fungal strains. Fungal strain no.WM-2 was selected and inoculated on 7 non-glutinous rice varieties in order to select a suitable rice variety for koji preparation.

After steaming, rice varieties with soft texture aggregated because of their high amylopection content. This aggregation affected fungal growth and enzyme productivity. As shown in Fig. 2, koji prepared from Leung11 and RD23 rice varieties had similar a-amylase activities. However, due to the above-mentioned fact that aggregated rice would adversely affect fungal growth and enzyme activity, Leung11 rice was selected for koji preparation because of its unaggregation characteristics.

Optimization of fermentation ratio between solid (ratio of koji to glutinous rice was fixed at 1:6.75) to liquid (35% (w/w) ethanol) was carried out using the total weight of mixture of 2 kg; the mixture was incubated for 60 days at room temperature. At an early stage of incubation, steamed rice adsorbed moisture from alcoholic solution and swelled. This was followed by gradual solubilization due to the action of koji enzymes. The liquid fraction was analyzed for reducing sugars, nitrogen and alcohol to monitor the process of solubilization of raw materials. Uchida and Oka (1983) reported that the concentration of reducing sugars in the liquid fraction reached 39% within 20 days of incubation. On the other hand, the alcohol concentration

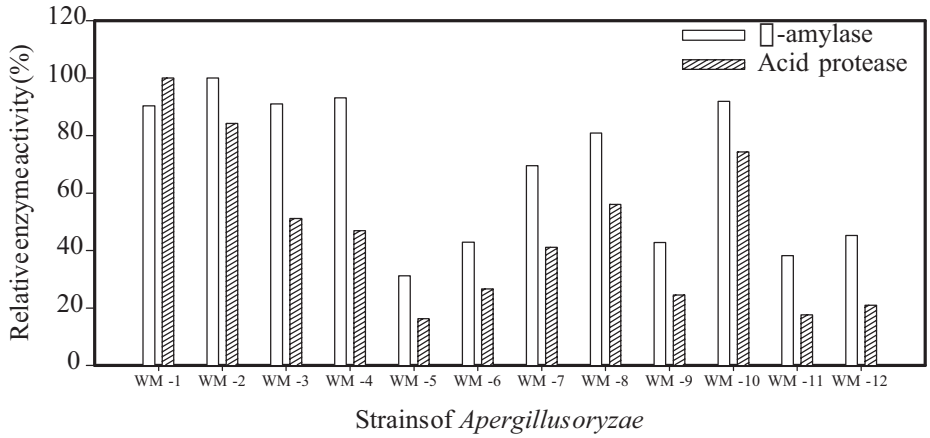


**Figure 1** Relative enzyme activities of a–amylase and acid protease from various strains of *Aspergillus oryzae*.

rapidly decreased from 35% to about 10% within 10 days of incubation.

Table 1 shows the analytical data of the product. The amount of reducing sugars obtained was 27-44 % (w/v) in all of the four formulas including the original one. The final amount of alcohol obtained was 9-16 % (v/v), depending on the solid:liquid ratios while the original formula contained the lowest amount of alcohol (< 10% (v/v)); formula 2 and 3 gave 13.23 and 16.06% (v/v) alcohol, respectively. Although formula 2 and 3 had less color intensity compared with that of the commercial mirin, formula 3 had the palest color and lowest amount of reducing sugars. Therefore, formula 2, which had acceptable amount of alcohol and pale color, was selected and subsequently used in the experiments, which would be carried out at the solid:liquid ratio of 60:40.

For the determination of the ratio between koji and glutinous rice, the ratio of solid:liquid was fixed at 60:40. The mash (2 kg) was prepared with 800 g of 35% alcohol (w/w) and various amounts of koji and glutinous rice

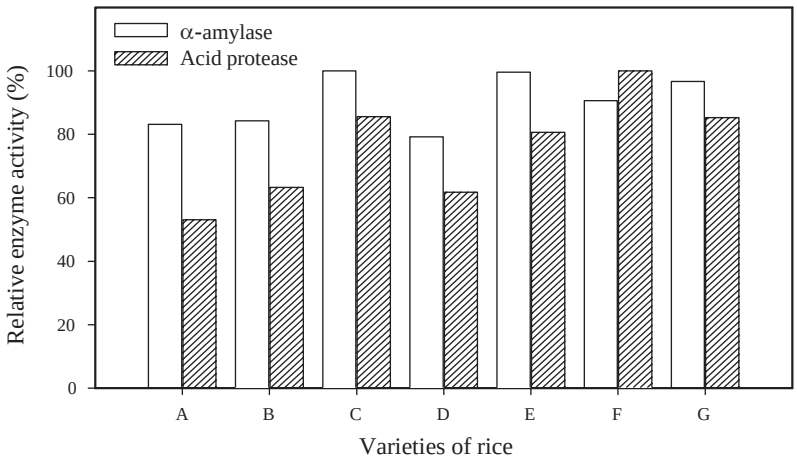


**Figure 2** Relative enzyme activities of *A. oryzae* strain no.WM-2 in various varieties of rice. (A: Supanburi 1, B: RD19, C: RD23, D: Khao Dawk Mali 105, E: Leung 11, F: Leung-aun, G: Chai Nat 1).

**Table 1** Chemical and physical properties of mirin at various ratios of solid to liquid. The ratio of Koji to gluinous rice in the solid fraction was fixed at 1:6.75.

| Formula | Ratio of solid:liquid[3/] | Chemical and physical property | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Total acid (% w/v) | pH | Reducing sugars (% w/v) | Soluble solids ( $^{o}$Brix) | Alcohol (%v/v) | Color ($A_{440}$) | Turbidity ($A_{660}$) |
| Com[1/] | - | 0.401$^{a}$ | 5.73$^{d}$ | 68.45$^{a}$ | 46$^{a}$ | 10.66$^{c}$ | 0.247$^{c}$ | 0.005$^{d}$ |
| ORI[2/] | 72.1:27.9 | 0.398$^{a}$ | 5.88$^{c}$ | 43.61$^{b}$ | 39$^{b}$ | 9.16$^{d}$ | 0.312$^{a}$ | 0.016$^{a}$ |
| 1 | 70:30 | 0.374$^{b}$ | 5.90$^{c}$ | 39.48$^{c}$ | 38$^{c}$ | 10.40$^{c}$ | 0.262$^{b}$ | 0.012$^{b}$ |
| 2 | 60:40 | 0.278$^{c}$ | 6.00$^{b}$ | 31.38$^{d}$ | 33$^{d}$ | 13.23$^{b}$ | 0.163$^{d}$ | 0.008$^{c}$ |
| 3 | 50:50 | 0.195$^{d}$ | 6.13$^{a}$ | 26.91$^{e}$ | 32$^{e}$ | 16.06$^{a}$ | 0.109$^{e}$ | 0.006$^{d}$ |

Note: Means in the same column followed by different superscripts are significantly different ($p<0.05$).

[1/] Commercial mirin, [2/] original formula (control) (Uchida and Oka, 1983) [3/] Koji and glutinous rice: 35% (w/w) alcohol

(koji/glutinous rice of 600/600 g, 400/800 g, 300/900 g, 200/1000 g, 150/1050 g and 120/1080 g). The ratios of the amount of koji to that of glutinous rice were set at 1:1 to 1:9.

Table 2 shows that the amount of reducing sugars obtained was 26-38 % (w/v) in all seven formula, including control, while the amount of nitrogen obtained was 0.07-0.22% depending on koji:glutinous rice ratio. The final amount of alcohol obtained was 9-15 % (v/v). It is noted from this table that formula E mirin had the amounts of reducing sugars of 37.5 % (w/v) and total soluble solid of 42.23 ∂Brix, which was most similar to the traditional mirin (Uchida and Oka, 1983). In addition, the nitrogen content of formula E was 0.086% (w/w) lower than that of formula D, which could help to improve the flavor and color of mirin. Therefore, formula E mirin, prepared using the koji to glutinous rice ratio of 1:7 and solid:liquid ratio of 60:40 gave the best quality and acceptable mirin as compared with the commercial mirin.

The results from the scaling up of koji production to the pilot scale showed that after 12 h of incubation growth of *A. oryzae* strain no. WM-2 was fast and led to the highest enzyme productivity and maximum growth between 24-48 h. During the stationary phase, *A. oryzae* no. WM-2 (36 h) continuously produced a-amylase while the protease productivity was stable (Fig. 3). Narahara *et al*. (1982) reported that at the stationary phase of growth, a-amylase activity still increased while protease as well as saccharifying activities were constant. Since air ventilation is necessary for the growth of fungus and enzyme production of koji, preparation of koji in the koji machine led to higher enzyme activity than doing so in the laboratory, which had no air circulation.

The results in Table 3 indicate that koji preparation in the koji machine with the bed depth of 1 inch gave the highest amount of a-amylase and protease activities. The condition in the koji chamber was suitable for fungal growth and

**Table 2** Chemical and physical properties of mirin at various ratios of koji to glutinous rice. Solid:liquid ratio of mixture was fixed at 60:40.

| Formula | Koji:Glutinous rice | Chemical and physical property of mirin | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Acidity (% w/v) | pH | Reducing sugars (% w/v) | Soluble solids ÐBrix | Alcohol (% v/v) | Nitrogen (%w/w) | Color ($A_{440}$) | Turbidity ($A_{660}$) |
| COM[1/] | - | $0.401^{a}$ | $5.73^{cd}$ | $68.45^{a}$ | $46.00^{a}$ | $10.66^{d}$ | $0.066^{e}$ | $0.247^{cd}$ | $0.005^{a}$ |
| Con[2/] | 1:6.75 | $0.193^{e}$ | $5.62^{ef}$ | $36.04^{c}$ | $43.67^{b}$ | $9.20^{e}$ | $0.138^{c}$ | $0.219^{de}$ | $0.017^{c}$ |
| A | 1:1 | $0.337^{b}$ | $5.58^{f}$ | $26.07^{f}$ | $37.60^{c}$ | $14.60^{a}$ | $0.220^{a}$ | $0.493^{a}$ | $0.028^{b}$ |
| B | 1:2 | $0.257^{c}$ | $5.67^{de}$ | $29.50^{e}$ | $39.73^{c}$ | $14.00^{a}$ | $0.180^{b}$ | $0.341^{b}$ | $0.019^{bc}$ |
| C | 1:3 | $0.222^{d}$ | $5.70^{de}$ | $33.00^{d}$ | $40.93^{d}$ | $12.50^{b}$ | $0.128^{c}$ | $0.211^{def}$ | $0.015^{c}$ |
| D | 1:5 | $0.188^{e}$ | $5.78^{bc}$ | $36.50^{c}$ | $41.60^{e}$ | $10.60^{d}$ | $0.125^{c}$ | $0.249^{c}$ | $0.020^{bc}$ |
| E | 1:7 | $0.154^{f}$ | $5.85^{ab}$ | $37.50^{c}$ | $42.23^{f}$ | $10.00^{d}$ | $0.086^{d}$ | $0.192^{ef}$ | $0.015^{c}$ |
| G | 1:9 | $0.142^{g}$ | $5.88^{a}$ | $37.00^{b}$ | $42.33^{g}$ | $11.60^{e}$ | $0.074^{e}$ | $0.179^{f}$ | $0.015^{c}$ |

Note: Means in the same column followed by different superscripts are significantly different ($p<0.05$).

[1/] Commercial mirin, [2/] Control

enzyme production. However, the rice thickness should not be more than 2 inches because the air ventilation would not be enough.

From the obtained ratio of raw materials for mirin-making, scaling up of mirin production to pilot scale (50 kg) involved the use of 3.75 kg of koji and 26.25 kg of glutinous rice mixed with 20 kg of 35% (w/w) alcohol. After termination step of mirin fermentation at pilot scale, the composition analysis of mirin showed that mirin

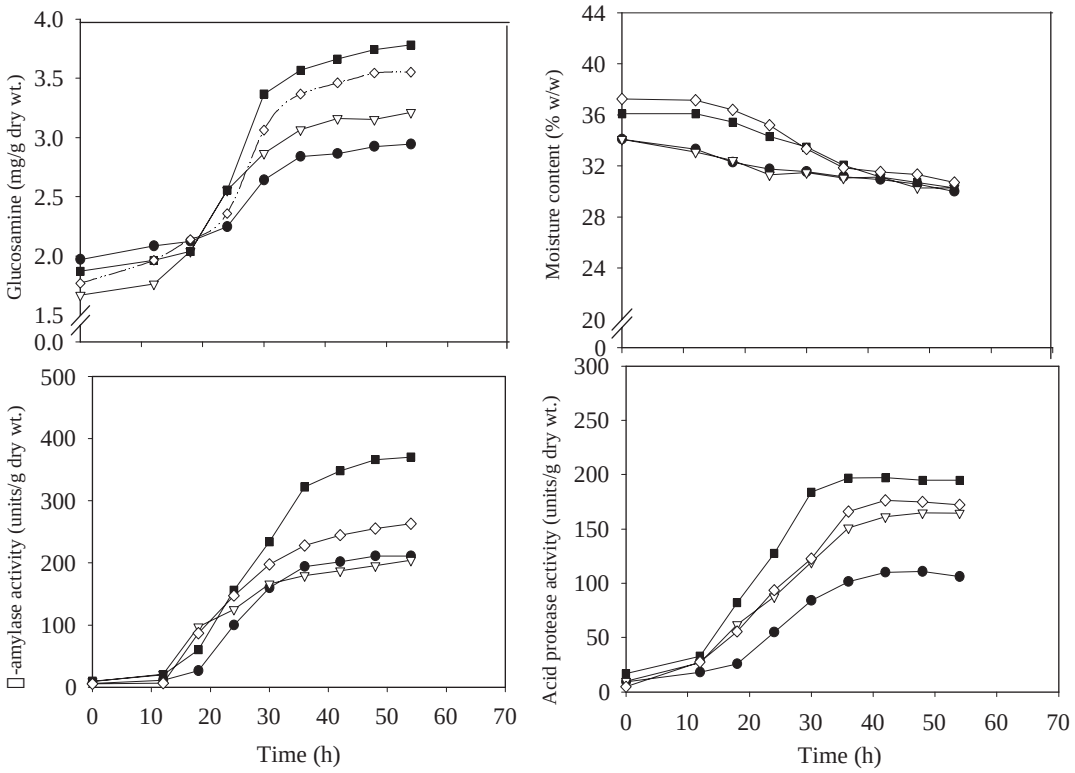


**Figure 3** Comparison of glucosamine level, moisture content and enzyme activities (a-amylase, acid protease activity) between laboratory scale and pilot-scale. Thickness of rice bed: (э) lab scale**,** (—) 0.5 inch, (■) 1.0 inch and (‡) 2.0 inch.

**Table 3** Effect of rice bed thickness on enzyme production of *A. oryzae* strain no. WM-2 using pilot-scale koji machine.

| Bed thickness (inch) | Rice weight (kg) | a-Amylase activity (units/g dry wt.) | | Acid protease activity (units/g dry wt.) | |
|---|---|---|---|---|---|
| | | At the highest level | At 36 h | At the highest level | At 36 h |
| 0.5 | 5.0 | 204.25$^{b}$ | 179.47$^{d}$ | 164.89$^{c}$ | 150.82$^{c}$ |
| 1.0 | 10.0 | 370.14$^{a}$ | 322.10$^{a}$ | 197.23$^{a}$ | 196.82$^{a}$ |
| 2.0 | 15.0 | 162.93$^{c}$ | 228.04$^{b}$ | 176.21$^{b}$ | 165.90$^{b}$ |
| Control[1/] | 1.0 | 210.97$^{b}$ | 193.74$^{c}$ | 110.68$^{d}$ | 101.45$^{d}$ |

Note: Means in the same column followed by different superscripts are significantly different ($p<0.05$).

[1/] Control experiment was carried out in laboratory, not in koji machine.

at the pilot scale had slightly higher total soluble solids, reducing sugars, acid, nitrogen amount and color intensity than those of mirin which was obtained at the laboratory scale. As shown in Table 4, the amounts of reducing sugars, acid and color intensity obtained at the pilot scale were lower than those of commercial mirin. Therefore, a sensory evaluation comparing the obtained mirin with the commercial one was performed.

Sensory evaluation results showed that commercial mirin, which had more reducing sugars and total soluble solid content than did formula E, was accepted more easily. However, the acceptable levels of color, flavor and overall likeness were not significantly different (Tables 4 and 5). For formula E mirin, consumer acceptability in terms of the overall likeness was moderate (5.66).

## CONCLUSION

Fungal strain no. WM-2, isolated from commercial spore of *Aspergillus oryzae* from Japan, showed the highest enzyme activities of a-amylase and protease productivity. The suitable non-glutinous rice variety for koji preparation was Leung 11 due to unaggregation of rice grains. Based on the optimization of fermentation ratio between solid and liquid, formula 2 mirin using 60:40 ratio gave the best quality of mirin with pale color and residual alcohol level. Mirin prepared using the ratio of koji to glutinous rice of 1:7 had similar levels of reducing sugars and total soluble solid content to those of traditional mirin. In addition, residual alcohol was not significantly different. Mirin with pale color was easily accepted by untrained panelists.

**Table 4** Comparisons of commercial mirin and formula E (pilot scale).

| Chemical and physical property | Commercial mirin | Formula E |
|---|---|---|
| Soluble solids (∂Brix) | $46.0^{a}$ | $43.5^{b}$ |
| Reducing sugars (%w/v) | $68.45^{a}$ | $41.05^{b}$ |
| pH | $5.73^{a}$ | $5.80^{a}$ |
| Total acid (%w/v) | $0.401^{a}$ | $0.184^{b}$ |
| Alcohol (%v/v) | $10.66^{a}$ | $10.5^{a}$ |
| Nitrogen (%w/w) | $0.066^{b}$ | $0.105^{a}$ |
| Color ($A_{440}$) | $0.247^{a}$ | $0.19^{b}$ |
| Turbidity ($A_{660}$) | $0.005^{b}$ | $0.010^{a}$ |

Note: Means in the same row followed by different superscripts are significantly different ($p<0.05$).

**Table 5** Mean of hedonic score for sensory attributes of mirin.

| Quality | Mean of hedonic score | |
|---|---|---|
| | Commercial mirin | Formula E |
| Color$^{ns}$ | 6.69 | 6.80 |
| Flavor$^{ns}$ | 5.23 | 5.74 |
| Sweetness | $6.29^{a}$ | $5.31^{b}$ |
| Overall likeness$^{ns}$ | 6.20 | 5.66 |

Note: ns= pΔ0.05 not significantly different.

Means in the same column followed by different superscripts are significantly different ($p<0.05$).

Scaling up of koji preparation using the koji machine with 1 inch of rice bed thickness showed the highest a-amylase and protease activity at 36 h of incubation. Chemical and physical analysis of mirin produced at the pilot scale indicated that appearances of mirin from pilot and laboratory scales were similar. For the results of sensory evaluation, untrained panelists moderately accepted mirin formula E with an average hedonic point of 5.66.

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