

Research Article

Promotion of safe winemaking practices using quantity comparison and methanol-reduction process for rice wine and whisky

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Abstract

Research was conducted to determine the quantity of methanol in liquor locally produced in nine different areas of Kamphaengphet Province. All samples were investigated for methanol content and, while quantities varied depending on the fermentation process, the amounts of methanol determined were statistically significant. Sample No.3 had the highest methanol content at 54.03 ppm and the average varied from 34.58 ppm to 47.23 ppm.

Concurrent with this study was an investigation of the role played by methanol by examining the quantities in starter culture which must be fermented for a specific period of time. The highest methanol level was detected in 2% starter culture, covering 5 fermentation periods and showing results of 7.31, 7.47, 7.36, 7.29 and 7.63 ppm respectively. Lower levels of methanol were detected in 1.5%, and 1% starter culture. The results showed a clear trend of increasing amounts of methanol; the longer it was fermented, the higher amount of methanol it gained. The appropriate distillation temperature was at 85°C examined from a fermented sample which had the highest level of methanol. Distillation time included 1, 2, 4, 6, 8, 10, 12 and 14 minutes, resulting in the detection of methanol as follows; 5.30, 11.07, 16.33, 20.58, 23.46, 25.27, 25.79 and 26.02 ppm respectively.

As a part of this research, a workshop was conducted for distillers on skill development and knowledge promotion for rice wine, rice whisky and safe wine manufacturer. This included a campaign to promote safer rice wine distillation process wherein alcohol content does not exceed 15%. An evaluation at the completion of the workshop indicated that 85.31% of the participants had gained a dimension of knowledge and understanding, 91.03% of the

participants had a dimension of satisfaction and 100% of the participants had a dimension of knowledge utilization.

Keywords: alcohol, distillation, starter culture, knowledge promotion, Thailand, training

Introduction

Although the current state of the local liquor became popular in Muang District, Kamphaengphet Province, the production was mostly done in household level. The quality of the liquor wasn't stable. In addition, a traditional liquor process had not been developed, or was under the control of the process as they should [1]. The most volatile matter obtained from distillation was ethanol and often contained other substances, for instance, methanol, fusel oil [2] and methanol was dangerous for humans. It could damage central nervous system, optical nerve and liver [3].

Moreover, there were many researchers who were interested in studying the chemical composition of liquor, for example, Kitiphong [4] analyzed liquor's main volatile matter and chemical composition which made from Thai rice. From 26 liquor samples, at least 28 kinds of chemical composition were found. Likit [5] analyzed chemical composition of 31 nationwide samples and detected at least 44 types of chemicals which consisted of ethanol, methanol, ethyl acetate, acetaldehyde, etc. Sombat [6] studied and designed a distilled liquor pot. He found that volatile temperature was between 78-85°C and kept the temperature below 85°C for the whole process, the alcohol quality would be suitable as a model for the alcohol production in community scale as well.

For the importance of the studies above, those results had an influence on the researcher to compare the volume of methanol and methanol reduction of liquor in Muang District, Kamphaengphet Province. It was expected that the results of this study could be applied in a further study. The knowledge of consumable liquor which was free from methanol added to the wine with low alcohol content less than 15% would be helpful to education, economics and other benefits. It could be applied for production in community scale and global wine industry.

Materials and Methods

Equipment

Gas Chromatography (Perkin Elmer brand Clarus 500 model made in Germany)
Micropipette (METTLER TOLEDO brand RAININ model made in Switzerland)
Volumetric Flasks (Size 50, 100, 250 and 500 ml).

Chemicals

Methanol HPLC Grade, made by Merck, Germany.
N-buthanol AR Grade, produced by Fluka, Germany.
Ethanol Absolute HPLC Grade, made by Merck, Germany.
Deionized Water.

Raw materials

Kor-Khor 6 glutinous rice from Nakornchum Market, Muang District, Kamphaengphet Province.

Starter culture (an amylolytic starter) from Nakornchum sub-district, Muang District, Kamphaengphet Province.

Solution preparation

1) Preparation of internal standard stock solution

Diluted 10 ml of N-butanol by 40 percent ethanol in the 100 ml volumetric flask

2) Preparation of methanol stock solution

Diluted 10 ml of methanol by 40 percent ethanol in the 100 ml volumetric flask

3) Preparation of 5 concentrated standard solutions from stock solution for illustrating standard curves consisted of 5, 10, 20, 40 and 80 ppm, respectively

Sample preparation

1) Liquor was filtered through filter paper No. 1

2) Put the part that stuck on the filter to the vial about 1 – 1.5 ml and closed tightly.

3) Determined the amount of methanol by a gas chromatography device

Gas chromatography device conditions

The gas chromatography device was Perkin Elmer brand Clarus 500 model. The conditions used were; Pressure 7 psi, FID Detector, Injector Temp 220°C, Oven Temp 45 – 120°C, Detector Temp 220°C, and Column Valcobond 30 m.

Experimental Methods

Part 1: Determination of methanol in distilled product

9 liquor samples were collected in Kamphaengphet Province and used 3-time analysis. The researchers modified the method of AOAC [7] by gas chromatography method, used n-butanol as an internal standard and adopted the Completely Randomized Design experiment in three times to compare mean values by Duncan's new Multiple Range Test.

Part 2: Determination of starter amount and the time spent on yeast fermentation

Glutinous rice was fermented by Starter culture for 5 days. The ratios of starter culture were 1, 1.5 and 2, respectively and then refill water at the 1:1 ratio of glutinous rice. After that left it for 1, 3, 5, 7 and 9 days, then the liquid was analyzed for the amount of methanol by using the method similar to the first part and adopted the factorial in Completely Randomized Design experiment in three times to compare mean values by Duncan's new Multiple Range Test for each sample.

Part 3: Effects of time on methanol in distilled spirit

The yeast contained most methanol from the second experiment by the distillation at temperature 85°C, the temperature commonly used in refining. During distillation was in process, the samples of liquor were collected at 1, 2, 4, 6, 8, 10, 12 and 14 minutes respectively to analyze the amount of methanol as in Part 1 by using the Completely Randomized Design experiment for three times. The mean values were compared by Duncan's new Multiple Range Test to determine how long it took to distill all of the methanol and to determine the appropriate time to produce suitable liquor for consumption.

Part 4: Performance of the methanol quantitative analysis

To evaluate the accuracy of the analysis by using a standard solution of methanol at the concentration of 40 ppm, μ l of substance 1 was injected for five times. Then analysis of the

data by a gas chromatography device and the retention time value with peak area value was calculated for RSD.

Part 5: Promoting knowledge of rice wine production and liquor for safe winemaking

To conduct a workshop for entrepreneurs and other interested persons with a knowledge test before and after attending the workshop. The knowledge gained from the research in Parts 1-4 would be transferred to participants and they would know how to increase alcohol safely with methanol by adding alcohol from rice wine (*Sa-to*). The workshop would be evaluated by using a questionnaire contained 3 directions which were acquisition, satisfaction and the knowledge to be exploited.

Experimental Results

Part 1: Determination of methanol in distilled product

The liquor produced in Kamphaengphet Province was collected amount 9 samples 3 times. The data were analyzed methanol volume as shown in Table 1.

Table 1. Determination of methanol in distilled product.

| Liquor sample | Volume of methanol (ppm) |
|---------------|--------------------------|
| 1 | 47.23 ^b |
| 2 | 41.92 ^c |
| 3 | 54.03 ^a |
| 4 | 41.07 ^c |
| 5 | 36.47 ^{d,e} |
| 6 | 34.58 ^e |
| 7 | 41.81 ^c |
| 8 | 36.56 ^{d,e} |
| 9 | 39.28 ^{c,d} |

Remarks: a, b ..., e were used as symbols to compare the difference of Level of Confidence at 95%.

Table 1 showed that methanol was found in every sample with varying quantities. The liquor sample 3 contained the highest amount of methanol at 54.03 ppm and the others had average amounts of methanol between 34.58 - 47.23 ppm.

Part 2: Determination of Starter amount and the time spent on yeast fermentation

The researchers found the amount of starter culture that affected the amount time spent on yeast fermentation as illustrated in Figure 1.

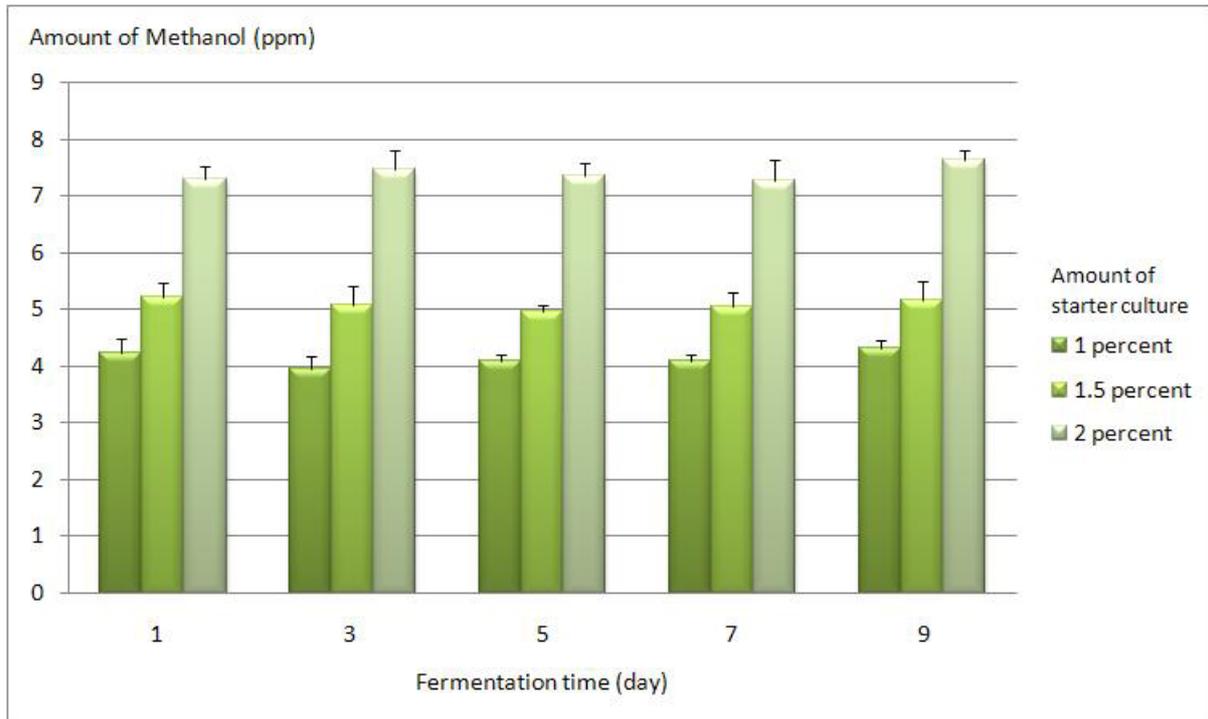


Figure 1. Effect of the amount of starter culture and fermentation time on the amount of methanol.

From Figure 1, it was found that the amount of the distilled spirit from fermented glutinous rice and starter culture had difference as shown in Figure 2, the ratios of starter culture 1, 1.5, and 2 per weight of cooked glutinous rice. The different fermentation periods of 1, 3, 5, 7 and 9 days (from the addition of water) were started from mixing starter culture with glutinous rice and fermented for 5 days and then adding water. The liquid from the process was analyzed for the amount of methanol and it was found that the liquor contained different amounts. In addition, statistical analysis showed that the duration of the fermentation did not affect the amount of methanol. It was only the amount of starter culture that had affect on the amount of methanol in yeast by spending 2% with methanol volume had the highest methanol at 5 times during fermentation which were; 7.31, 7.47, 7.36, 7.29 and 7.63 ppm, respectively, followed by the volumes of starter culture 1.5 and 1%.



Figure 2. Glutinous rice and starter culture. (A) mixture of glutinous rice and starter culture (B) glutinous mixed with starter culture after water addition.

Part 3: Effects of time on methanol in distilled spirit

The liquor sample that contained the highest volume of methanol was from fermentation of starter culture 2%. On day 4 of water addition, the sample was tested for the effects of time to distilled spirits per the amount of methanol. It was distilled at 85°C. As shown in Figure 3.

The result showed that liquor with the greatest amount of methanol was distilled to learn how long it took to make the liquor could be consumed at 85°C [8]. Liquor at different distillation had different amounts of methanol by statistical significance at the confidence level of 95% compared to average. The liquor obtained in the first minute had methanol volume at 5.30 ppm and in the place of 2, 4, 6, 8, 10, 12 and 14, the amounts of methanol were 11.07, 16.33, 20.58, 23.46, 25.27, 25.79 and 26.02 ppm, respectively. It likely increased until the minute 10. After that the amount of methanol is relatively constant. That meant methanol had gone out. By comparing the amount of methanol and ethanol distilled from the yeast at 10 minutes, the methanol volume was not different, whereas for the ethanol, it increased significantly.

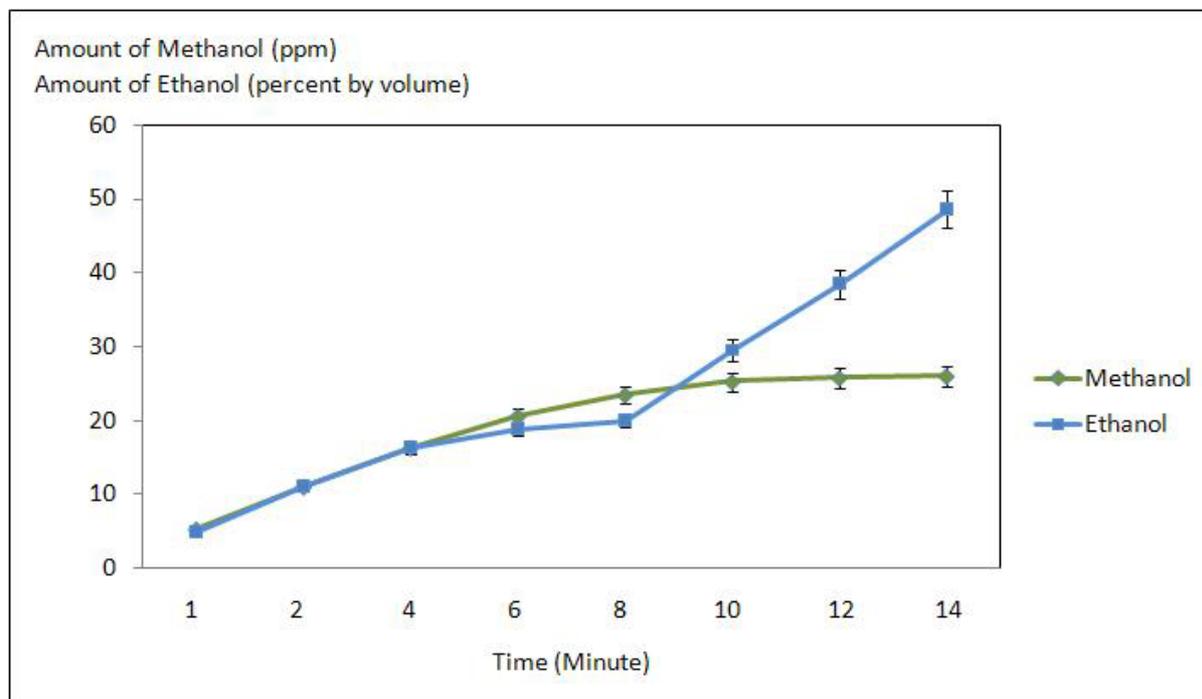


Figure 3. Effect of duration in distilled spirits on methanol volume.

Part 4: Performance of the methanol quantitative analysis

The efficacy of quantitative analysis of methanol volume in distilled spirits from sample preparation to equipment conditions was studied and the results are shown in Table 2.

Table 2. Retention time value and peak area value for accuracy.

| Time | Retention time (min.) | Peak area |
|----------|-----------------------|-----------|
| 1 | 2.89 | 181.97 |
| 2 | 2.88 | 182.15 |
| 3 | 2.87 | 181.51 |
| 4 | 2.86 | 181.68 |
| 5 | 2.85 | 181.82 |
| Mean | 2.87 | 181.82 |
| S.D. | 0.016 | 0.50 |
| % R.S.D. | 0.56 | 0.28 |

From Table 2 it was found that the precision of the five-time experiment by using the liquor example 3 was analyzed by a gas chromatography device. Then the retention time value and the peak area value were calculated for RSD. The RSD of the retention time value and peak area value was reported at 0.56 and 0.28%, respectively, which were lower than standard value. Normally, the RSD of the retention time value and the peak area value might not exceed 1 and 2%, respectively. The result showed that the gas chromatography device worked with high accuracy.

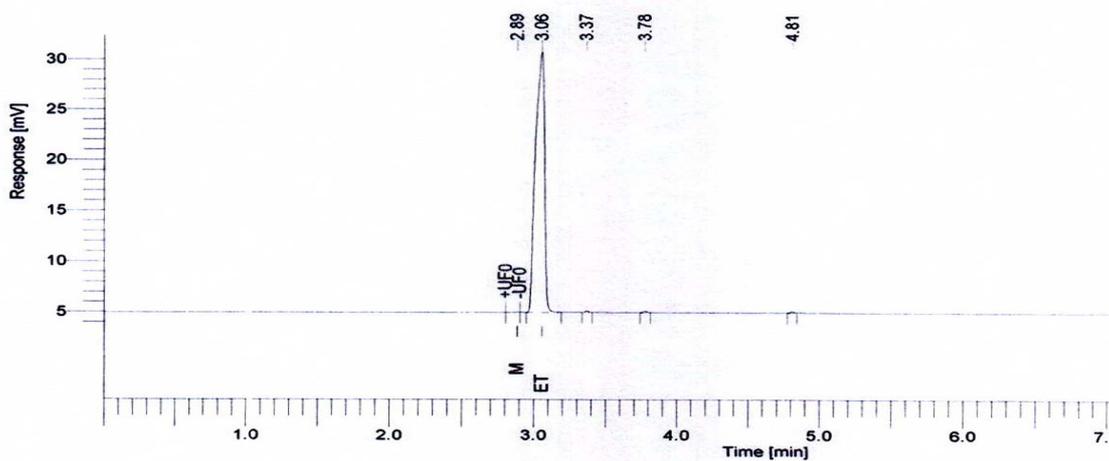


Figure 4. Chromatogram illustrating degree of methanol in rice wine.

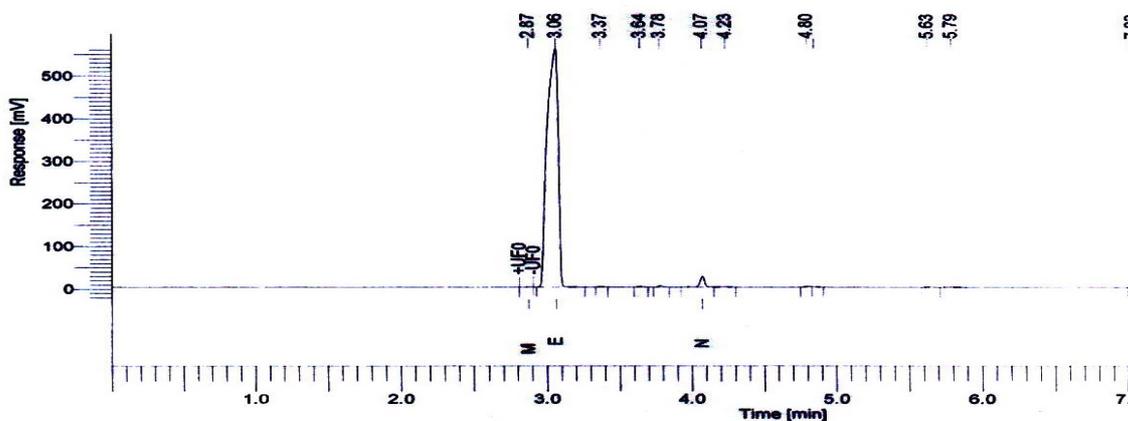


Figure 5. Chromatogram illustrating degree of methanol in liquor.

Part 5: Promoting knowledge of rice wine production and liquor for safe winemaking

The workshop for entrepreneurs and other interested persons had 34 participants consisting of 28 women representing 82% and 6 men representing 18%. Most of them (18 people) were at the age of above 50 representing 53% and most of the participants (26 people) had education background below bachelor degree at 76%.

Percentage of the knowledge and understanding of manufacturing processes for the production of rice wine (*Sa-to*) and liquor of the workshop participants were increased from 65% to 85%, representing a 20% increase.

Percentage of satisfaction towards the workshop were at extremely satisfied level with 4.55 of mean value and representing 46%, at highly satisfied level 35% and 17% for moderately satisfied level. The average satisfaction was 91%.

Percentage of the knowledge gained and applied from participating in the workshop was 100% and no participants thought that they could not apply the knowledge.



Figure 6. Activities to promote the knowledge of safe rice wine and liquor production.

Results and Discussion

For Part 1, liquor samples contained methanol in different volume levels, which is still considered acceptable by the community product standard (Liquor Product). It is allowable to have 420 ppm of methanol [9]. Because the analyzed liquor was made of sticky rice or molasses, the volume of methanol was very low [8]. However, although the amount of methanol was acceptable, it could be harmful for extreme consumption as well.

Part 2 showed that the volume of methanol was different. The duration of the fermentation of methanol had no effect on the amount of starter culture, but volume of starter culture had an effect on the amount of methanol in yeast. Utilizing 2% of starter culture produced the highest amount of methanol at 5 periods of fermentation which were at 7.31, 7.47, 7.36, 7.29 and 7.63 ppm, respectively, and followed by spending starter culture at 1.5 and 1 %. The cause of methanol rising was not from the fermentation process. It was caused by the methyl ester enzyme pectin hydrolyzed bond of the methyl ester of pectin so pectin acid and methanol were produced [10].

Our research indicated that methanol was formed before the fermentation process. The enzyme pectin was most commonly found in multi-cellular plant as pectin compound but in

different layers of cells. Most were found in fruit and vegetables [11]. Therefore, the amount of methanol was less. Besides, steamed rice at high temperature for long period of time could destroy the enzyme [12]. When it was compared with the amount of ethanol, liquor had a tendency of higher amounts of ethanol from longer fermentation period. The samples were 1.5% of starter culture in the fermentation period of 9 days, the highest average amount of ethanol was 15.15% and the average ethanol volume was from 3.64 to 8.60%.

The third experiment showed that the amount of methanol was relatively constant when the distilled liquor was at minute 10. The volumes of methanol were indifferent but ethanol increased significantly. Because the distillation of alcohol was based on its ability to evaporate so the volatile substance was evaporated before weighty substance. Methanol had a boiling point of 64.7°C and the boiling point of ethanol was 78.5°C [13]. It was shown that methanol had a lower boiling point than ethanol. Methanol was removed before ethanol.

When comparing the amount of methanol and ethanol in distilled liquor at minute 10, the methanol content showed no difference but the ethanol content increased. Thus, we should collect the liquor for consumption in the 10th minute onwards for the safety of consumers. In addition, a while after distilled liquor process, methanol concentration was not very constant because it partially stuck at the equipment used in refining. The solution was to design, or to use the equipment which could be cleaned to remove the toxins easily. In particular, condensation was very important to get a better liquor quality.

In our experiments, Section 4, the analysis showed the performance of methanol in distilled spirits. The study found the rate of recovery of methanol from the sample preparation. The return value of methanol, which was the average of 98.80%, was close to 100%. The value showed effective equipment and sample preparation provided a great value.

Section 5 of the experiment showed that participants who attended the workshop of promoting knowledge of *Sa-to* and liquor for safe wine production gained knowledge and were satisfied with the activities. They can adopt this knowledge in the manufacture of alcoholic beverages in the future. It would bring benefits to economy and society.

Conclusion

Rice wine (*Sa-to*) and liquor have been bound to Thai lifestyle for a long time. *Sa-to* and liquor drinking were popular in Thais especially during festivals. People in Kamphaengphet Province had also commercialized a large amount of liquor. The results of the study could be applied to those who actually wanted to produce liquor and *Sa-to* with less methanol, especially for household industry to provide more secure product. This study can be applied for product with low alcohol by adding liquor obtained from the process. It was safe for consumption.

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