



## **Simplification of the Colorimetric Method to Detect Methanol Contamination in the Cambodian Local Rice Liquor**

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**Abstract** Methanol, an alcohol, is known to be hazardous for human consumption. Methanol contamination in traditional rice liquor caused many deaths in local areas in Cambodia. Contamination happened in every step of liquor manufacturing, distribution, and consumption. To avoid this problem, monitoring the quality of alcohol is important. However, only a few government institutes in the capital can detect the methanol contamination at an institutional level by colorimetric methods. To detect methanol contamination easily at the local level, a simplified method is urgently required. We tested the original colorimetric methods to determine the influence of the amount of chemical solutions, the time and the alcohol percentage to the color change. Further we checked the shelf life of the chemical solutions. The results showed that methanol was detectable at one-twenty of original volume after treatment 2-5 hours, and the alcohol percentage was not influence of the color changes. In addition, we tested 21 liquor samples collected from markets in Phnom Penh and 6 provinces with the simplified method, resulting that methanol was not detected in all samples.

**Keywords** local liquor, methanol, simplified methods

### **INTRODUCTION**

Methanol, an alcohol, is known to be hazardous for human consumption. In the worst-case scenario, symptoms of methanol poisoning caused by inhalation or intake are blindness and death (Bennet et al. 1953). Recently, newspapers have reported that the contamination of methanol in traditional rice liquor caused many deaths in local areas of Cambodia (Gee & Martin 2012, The Cambodia Daily 2011). People bought cheap industrial alcohol at a high concentration, and mixed it as a loading fluid in every step of liquor manufacturing, distribution, and consumption.

Usually, when the equipment is available, the detection of methanol contamination is performed by gas chromatography, which is a quick and accurate method. However, due to high cost and shortage of human resources, this might be difficult to perform in developing countries.

In Cambodia, the Industrial Laboratory Center of Cambodia and CAMCONTROL in Phnom Penh have the resources to monitor the quality of alcohol at an institutional level using colorimetric methods. However, government agencies for the control of the quality of alcohol have not been established in

other provinces. Therefore, the establishment of a simple method to detect methanol contamination based on the existing protocol is required promptly to decrease the number of fatal accidents occurring with traditional rice liquor in Cambodia or other developing countries.

## **OBJECTIVES**

In this study, we improved some aspects of the conventional colorimetric method for detecting methanol by visual analysis. In order to simplify the colorimetric method, we aimed to modify the following 4 aspects: (1) detection sensitivity depending on reduced amount of chemical solutions, (2) optimum time to check the solution color after the final treatment, (3) detection sensitivity depending on the alcohol percentage, and (4) retention period of the chemical solutions. In addition, we conducted (5) methanol detection in rice liquors available in the local markets from 6 provinces.

## **METHODOLOGY**

### **Original Method**

We followed the colorimetric method reported by the Japan National Tax Agency in 1961 (National Tax Agency of Japan 2007). A brief description of the method is reported below.

**Preparation of chemical solutions:** Solution A (500 mL): 15 g of potassium permanganate (VII) ( $\text{KMnO}_4$ ) and 75 mL of phosphoric acid ( $\text{H}_3\text{PO}_4$ ) were dissolved in distilled water and up to 500 mL, Solution B (500 mL): 25 g of oxalic acid dihydrate [ $(\text{COOH})_2 \cdot 2\text{H}_2\text{O}$ ] was dissolved in 500 mL 50% (v/v) sulfuric acid ( $\text{H}_2\text{SO}_4$ ), Solution C (500 mL): 0.5 g of basic fuchsin was dissolved in about 300 mL of distilled water boiled at  $100^\circ\text{C}$ , and 5 g of sodium sulfite ( $\text{Na}_2\text{SO}_3$ ) was dissolved in about 50 mL of distilled water. Fuchsin solution was mixed with  $\text{Na}_2\text{SO}_3$  solution and 5 mL of hydrochloric acid (HCl) was added. Then, the final solution was diluted to 500 mL with distilled water. Finally, 40 mL of 10N  $\text{H}_2\text{SO}_4$  was added, and the solution was kept at room temperature for more than 5 h. The color often became light brown after adding HCl. Although it should be used after the color of fuchsin disappear, it could be a pale yellow color solution. The all chemicals were purchased from Wako Pure Chemical Industries.

**Preparation of standard solutions:** The standard solutions were prepared with 99.5% ethanol and methanol (Wako Pure Chemical Industries, Ltd). Ethanol was adjusted to 40% (v/v) with distilled water, and 0, 5, and 15  $\mu\text{L}$  methanol was added into 10 mL of 40% ethanol to make the 0, 0.05, and 0.15% (v/v) methanol artificial contaminated solutions since the Cambodian government set 0.15% is the highest standard level of methanol contamination in the original liquor. Finally these mixtures were 8 times diluted to prepare the 5% alcohol percentage for testing.

**Test procedures according to original method:** The sample solution (5 mL) was mixed in a test tube with 2 mL of solution A and left for 10 min. Then, 2 mL of solution B and 5 mL of solution C were added in this order and mixed well. After 30 min, the color of the solution was checked and the absorbance was determined at 590 nm with a spectrophotometer (U-2000A, HITACHI) (Hayashibe 1955) using low volume cell. Checking the absorption wavelength of standard solution determined the peak at 580-600 nm, hence we adopted 590 nm as the wavelength for the measurement. The colors of mixture turned from clear and colorless (0%), weak purple (0.15%) to purple (over 0.8%) according to the trial experiment.

### **Simplification of the Original Method**

The 40% ethanol with methanol 0, 0.05 and 0.15% (v/v) was diluted into 5% as the standard solutions. All steps were conducted under the room temperature (about 22-25°C).

**Dose reduction of the chemicals:** Various total amounts of standard and chemical solutions (i.e., 1, 1/2, 1/5, 1/10 and 1/20 of the amount indicated in the original protocol) were used to detect the methanol contamination in standard solutions. The color of the solution was assessed by visual analysis and spectrophotometer (590 nm) in following analyses.

**Optimum time to check the color of the solution:** The change of color was assessed 0.5, 1, 2, 3, 5, and 24 h after adding the final solution C. The amounts of solution were as follows: standard 0.25 mL, solution A 0.1 mL, solution B 0.1 mL, and solution C 0.25 mL.

**Difference in the sensitivity depending on the percentage of alcohol in the sample solution:** We compared the sensitivity of the colorimetric method between ethanol standard solutions (5%) and original solutions (40%) respectively. Further, rice liquor samples produced in Cambodia (Sraa Takeo 40% alcohol/volume, Royal University of Agriculture, Phnom Penh, Cambodia) were used; original percentage of the liquors (40%) with 0, 0.05 and 0.15% methanol artificial contamination, and its 5% diluted solutions. The amounts of solution were as follows: ethanol/liquor solutions (40% or 5%) 0.25 mL, solution A 0.1 mL, solution B 0.1 mL, solution C 0.25 mL. The color was checked 3 h after treatment.

**Expiration period of chemical solutions:** Test solutions were kept for 2 and 4 weeks at room temperature (about 22–25°C) and, then, were tested in comparison with fresh solutions. The combination of the solutions is shown in Table 1; N: fresh solution and O: 2 or 4 weeks old solutions. The amounts of the solutions were as follows: standard solutions 0.25 mL, solution A 0.1 mL, solution B 0.1 mL, and solution C 0.25 mL. The color was checked 3 h after treatment.

**Table 1 The combinations between fresh and old solutions**

Test solution	Combinations*							
	1	2	3	4	5	6	7	8
A	N	O	N	N	O	O	N	O
B	N	N	O	N	O	N	O	O
C	N	N	N	O	N	O	O	O

\* N: fresh solutions, O: 2 or 4 weeks old solutions

**Detection of methanol from the local rice liquors:** Rice liquors collected from the local market in Phnom Penh and 6 provinces (Battambang, Kompong Chhnang, Prey Veng, Pursat, Svay Rieng, Takeo) were used as liquor samples; 3 samples/province and 21 samples in total. Rice liquors were clear and colorless and the original liquors were diluted into 5% for testing. The amounts of the solutions were as follows: liquor 0.25 mL, solution A 0.1 mL, solution B 0.1 mL, and solution C 0.25 mL. The color was checked 3 h after treatment.

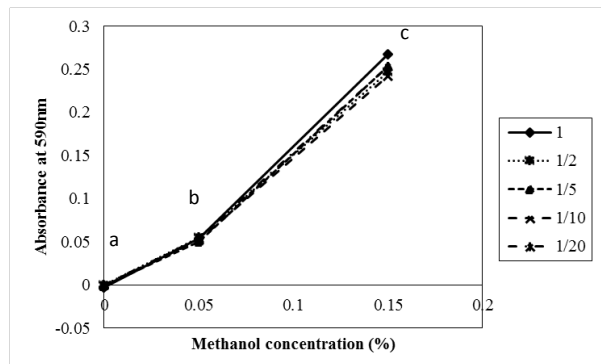
**Data analysis:** The data was analyzed using with Excel statistical analysis add-in software (Excel statistic 2012; Social Survey Research Information Co., Ltd., Tokyo, Japan).

## RESULTS AND DISCUSSION

The methanol percentage in the all figures indicated the methanol percentage in the original concentration (0, 0.05 and 0.15%), and the absorbance values of solutions (original/5% diluted) were shown without change.

**Scale-down of the amount of chemical solutions:** The reduction of the waste chemical solutions is required because the disposal of the toxic waste in Cambodia is not yet well-implemented and heavy metal such as  $\text{KMnO}_4$  pollutes the environment when discarded without treatment. We could recognized the difference between 0 and 0.15% methanol contamination by eyes in every total amount,

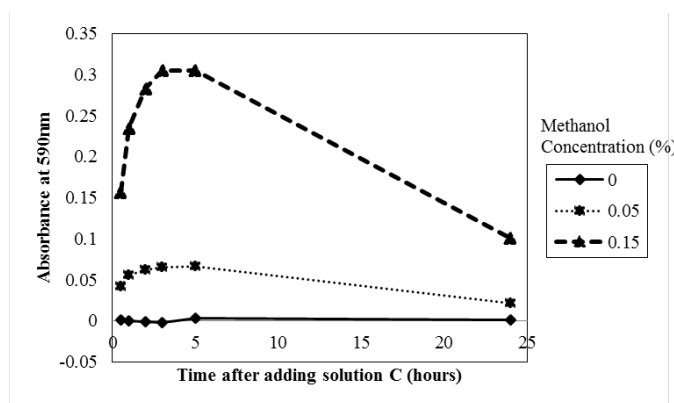
and the absorbance among 0, 0.05, and 0.15% methanol were significantly different in 1/20 of the amount in the original protocol (Fig. 1). It shows that methanol contaminations in the smallest amount of chemical solutions (ethanol standard: 0.25 mL, A: 0.1 mL, B: 0.1 mL and C: 0.25 mL) could be detected as same as original amount.



**Fig. 1 Scale-down of the amount of chemical solutions**

Significant differences among each methanol concentration of 1/20 of the initial solution were determined by one way-ANOVA. The same letters (a-c) mean no significant difference ( $p < 0.01$ ).

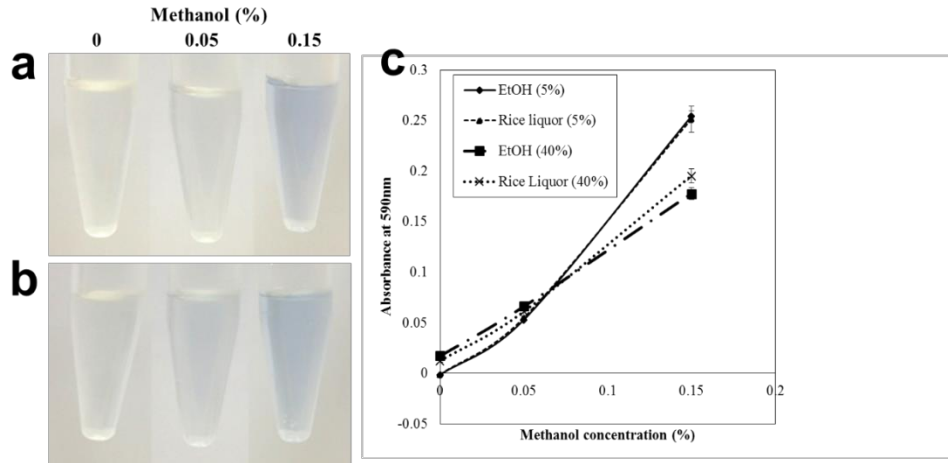
**Optimum time for detecting the color of the solution:** The optimum time for comparing the color by visual judgment was determined. 0.15% methanol, the highest allowed level of methanol contamination decided by the Cambodian government, was visually detected 0.5 h after adding the final solution. Two hours later, the differences of the color between 0% and 0.15% methanol could be observed easier, while no difference could be detected visually between 0% and 0.05% samples. However, after 24 h, although the difference of absorbance was detected by spectrophotometer, no discernible difference could be observed. Therefore, we can check the color of the solutions 2–5 h after treatment (Fig. 2).



**Fig. 2 Optimum time for detecting the color of the solution**

**Detection sensitivity for methanol contamination depending on the alcohol percentage:** The detection sensitivity of the 40% ethanol/rice liquor were compared with 5% diluted ethanol/ rice liquor to know whether methanol contamination can be detected in the original percentage. We could not recognize visually the difference between 0 and 0.05% methanol in both samples, however between 0% and 0.15%, it was detectable the difference by naked eyes though the absorbance was lower in original percentage than in 5% diluted one of both ethanol and liquor solutions (Fig. 3). To detect the accurate amount of methanol in alcoholic beverage, though gas chromatography is so convenience

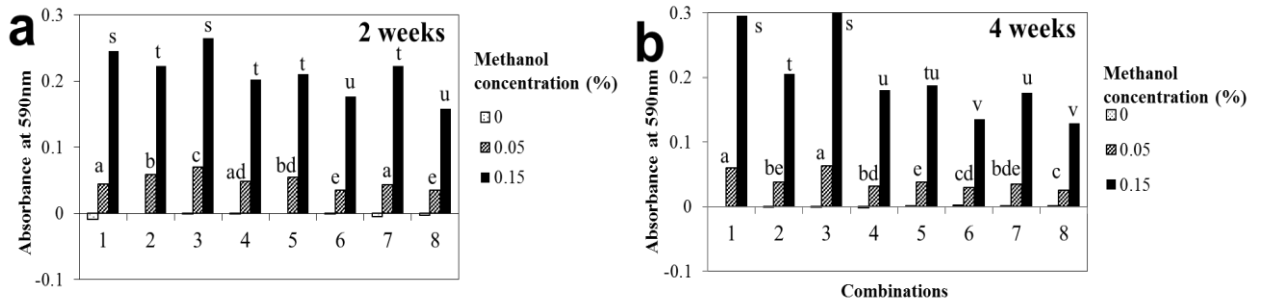
(Wang et al. 2004), the method shown in this study makes us possible to quick detection and visual discrimination if concentration of methanol included in the beverage is higher than specified safety level without using special equipments.



**Fig. 3 Difference of detection sensitivity for methanol contamination depending on the alcohol percentage**

Concentration-dependent change of solution color of (a) ethanol standard (5%) and (b) rice liquor (40%) with 0, 0.05, and 0.15% methanol contaminations from left to right, (c) concentration-dependent change of absorbance among 0, 0.05, and 0.15% methanol contaminations in 5% and 40% ethanol/rice liquor. The same letters (a-c) mean no significant difference ( $p < 0.01$ ) of the 40% rice liquor among each methanol concentrations as determined by one-way ANOVA.

**Expiration period of the chemical solutions:** To save time for the preparation of the chemicals, the retention period of chemical solutions was determined. The combination of fresh chemicals with old solutions was tested at 2 and 4 weeks after preparation. The detection sensitivity of the chemical solutions was not changed after 2 weeks. However, after 4 weeks, a difference could be observed and the detection sensitivity became lower in conditions 2, 4, 5, 6, 7, and 8, which include old A and C solutions. On the other hand, in condition 3, which includes old B solution, the color was the same as that of the normal fresh condition (Table 1, Fig. 4).



**Fig. 4 Expiration period of each chemical solution affecting detection sensitivity**

Dot, diagonal line, and black bars represent the absorbance of the 5% ethanol solution containing 0, 0.05, and 0.15% methanol, respectively. Conditions 1-8 show different combinations with fresh and old chemicals of A, B, and C (Table 1). Significant differences among conditions in (a) fresh and 2-week-old solutions, (b) fresh and 4-week-old solutions were calculated with one-way ANOVA respectively. The same letters mean no significant difference ( $p < 0.05$ ): 0.05% (a-e) and 0.15% methanol (s-v) among condition 1-8.

**Detection of methanol from the local rice liquors:** The 21 local rice liquor samples were tested by the simplified method. No methanol contamination was detected from those samples.

**Table 2 Detection of methanol contamination from local rice liquors**

Province	Number of sample	Methanol contamination
Battambang	3	Not detected
Pursat	3	Not detected
Kompong Chhunang	3	Not detected
Phnom Penh	3	Not detected
Prey Veng	3	Not detected
Svay Rieng	3	Not detected
Takeo	3	Not detected
Total	21	

## CONCLUSION

Based on our observations, we conclude that: (1) the amount of solutions can be reduced to a total volume of 0.7 mL (standard 0.25 mL, solution A 0.1 mL, solution B 0.1 mL, and solution C 0.25 mL); (2) the difference of color can be detected easily 2-5 h after the final treatment; (3) Moreover, the methanol contamination was detected without dilutions, and all the degrees of methanol contamination could be identified by comparison with 0% methanol standard solution though the detection ability was higher in 5% diluted solutions; (4) The degradation of the solutions increased with time, especially for solution C; therefore, the results indicated that solutions A and C could be used for 2 weeks, and solution B for 4 weeks. It is preferable to prepare fresh solutions within 2 weeks considering that the volatile substances in the solutions are hazardous; (5) Furthermore, this method can be used for rice liquor from local markets. Our results suggested that the simplified methods could identify the methanol contamination over the highest standard level by visual though it couldn't be measured accurate percentage of methanol contamination. Once the contamination is detected, the rice liquor is required to be analyzed in detail by laboratory experiments.

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