

## Determination Of Formaldehyde In Tofu From Ciputat Traditional Market With Colorimetry Method

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### Abstract

Tofu, a food product made from soybean, contain water and it is very easy to overgrown microbes. Based on a survey, many products of tofu contain formaldehyde as preservative. Formaldehyde is a preservative that the use is prohibited for food because it can cause cancer in humans. The aim of this study was determined concentration of formaldehyde in tofu from traditional market Ciputat using Spectrophotometer UVI-VIS. Steam distillation was used for sample extraction process. Distillate was reacted with Nash reagent. The equation of calibration curve is  $y=0,0032x - 0,0079$  and correlation coefficient of the linear regression of 0,9992. The experimental results obtained LOD value of 11.1328  $\mu\text{g/ml}$ , LOQ value of 37,1094  $\mu\text{g/mL}$ . The precision of this analytical method were lower than 2% for each of the sample, while method's accuracy for tofu was  $98,69\% \pm 0,4085\%$ . Results of sample analysis from Ciputat market, samples were detected containing formaldehyde. Formaldehyde concentrations are 104,87  $\mu\text{g/mL}$ , 11,21  $\mu\text{g/mL}$ , 1,96  $\mu\text{g/mL}$ , 190,80  $\mu\text{g/mL}$ , 201,98  $\mu\text{g/mL}$ , 10,47  $\mu\text{g/mL}$ , and 3,31  $\mu\text{g/mL}$ .

Keywords: Formaldehyde, Spectrofotometry UV-Vis, Tofu, Nash reagent.

### 1. INTRODUCTION

Formaldehyde,  $\text{CH}_2\text{O}$  is colorless tang gas that can be used as stabilizer with 15% metanol. (Dir. Jen. POM., 2003 ; Winarno, 2007). An aqueous solution of formaldehyde can be useful as a disinfectant as it kills most bacteria, fungi and viruses. It is also used as a preservative in cosmetics and a nail hardener in a concentration  $<1\%$ . Formaldehyde preserves or fixes tissue or cells and used as embalming agent. (Dreisbach, 1982).

A number of surveys and laboratory examination found that several food product used formaldehyde as preservative. Some of them circulated in traditional market are salted fish, fresh fish, wet noodles and tofu ( Dir Jen POM, 2003). Formaldehyde is used to extend storage time. The interaction of formaldehyde and protein in food produces a solid elastic texture of the food like wet noodle, fresh fish and tofu. Many consumer like this texture of the foods. The smell of food containing formaldehyde is hated by fly and they don't want

to perch. This longer storage time can provide benefit for both producer and consumer. The high price of diesel fuel and ice blocks are other reasons why sailors use formaldehyde as preservative for their fresh fishes. (Suwahono, 2009).

Formaldehyde can be toxic and hazardous for human. The toxic effect will not be appeared directly but manifested after years after the concentration of formaldehyde is accumulated in the body. Fatal dose of formaldehyde is 60 – 90 mL (Dreisbach, 1982). Tolerated dose for human body is 0.2 mg/kg body mass. (Anonim, 2006 ; Dir. Jen. POM., 2003).

BPOM (Drug and Food Inspection State Agency) has issued warning no. KH.01.04.53.094 on 24 July 2007 about Chinese food product imports and domestic food products that contain hazardous substances. BPOM has taken sample at some of markets and has conducted test of formaldehyde. The test has found out that these food products contain formaldehyde. Previous

testing of 700 food products from Java, Sulawesi and Lampung conducted in 2002 is founded that 56 % of sample contain formaldehyde.

There are 3 laws prohibit the use of formaldehyde, first UU no 7/1996 about food and UU no 8/1999 about consumer protection. The second one, minister of health regulations no. 1168/Menkes/PER/X/1999 states formaldehyde is a prohibited food additives. The thidr one, government regulations about regarding food safety dan nutritional quality no 28 on 2004 ( Depkes RI BPOM 2003).

Based upon those regulations, we conducted the research about determination formaldehyde content in tofu that circulated in Ciputat Market. Those tofu are suspected contain formaldehyde as preservative. We determined Ciputat market because it near from UIN Syarif Hidayatullah Campus and it is one of the big traditional market in Jakarta so that this research is purposed as UIN contribution to people around campus.

Determination of formaldehyde contain in sample is conducted by colorimetry method, with Nash reagent which contain ammonium acetate, glacial acetic acid, acetyl acetone and water, as the reagent. As we know formaldehyde with acetyl acetone will condense to form a yellow 2,6-dimethyl-3,5-diacetyl-1,4-dihydropyridin compound. This compound has green fluorescence color that can be detected in visible wave at 412 nm.

## 2. METHODOLOGY

### Instruments

Squash, distillation equipment, gas distillation equipment, measuring cups, erlenmeyer, thermometer, test tube, watch glass, metal spatula, analytical balance, aluminium foil, disposable gloves, masks, petri dish, volumetric pipette, waterbath, UV-Vis spectrophotometer.

### Material

Formalin (Merck) 37% (water solution of formaldehyde), Ammonium acetate (Merck), Acetic acid glacial (Merck), acetyl acetone

(Merck), solution of 10% phosphoric acid (Merck), soy bean, Calcium Sulphate/Sioko, Aquadest.

### Sample

The samples are tofu from Ciputat traditional market that are taken randomly.

### Preparation of Tofu simulation

Soybeans as much as 1 kg was washed by water, then soaked with water for approximately 5-6 hours. Soybeans crushed using a blender with added of warm water (soya and water ratio is 1:2). Soybeans that was crushed was warmed to 70-80°C in the waterbath and filtered. Filtrate was warmed again and added CuSO<sub>4</sub> until the blob was formed. Tofu is ready for the compacted.

### Measure of maximum wavelength.

A solution of 1 ml formaldehyde 150 ppm inserted to test tube and adjusted with water until 10 ml. The solution added Nash reagent 5 ml, and then kept at 37°C in waterbath for 30 minutes. Mixtures moved to volumetric flask 25 ml and adjusted with water until 25 ml, and shaken until homogeneous. Maximum wavelength was measured by UV-Vis spectrophotometer.

### Making Calibration Curve and Measured Linieritas.

Making calibration curve is done by methods that was same as measure of maximum wavelength. Absorbance of solution of formaldehyde on a series of concentration was measured at the maximum wavelength.

### Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection was calculated using the formula

$$LOD = \frac{3.3(Sy/x)}{b}$$

The Limit of Quantification was calculated using the formula:

$$LOQ = \frac{10 Sy/x}{b}$$

Sy/x is standard deviation

b is slope of linier regression.

### Accuracy and Precision

Tofu simulation crushed in mortar and then added formaldehyde concentration of 125 ppm and inserted into the distillation flask. Into the distillation flask added 100 ml water and 10 ml of 10% phosphoric acid and then were distilled steam. Distillate collected into a 100 ml erlenmeyer which already contains 10 ml of water (end of the pipe dipped into distilled water) and were distilled until the distillate is obtained until a boundary. It was covered with aluminum foil and shake until a homogeneous. Then, concentration of formaldehyd was measured. Performed the same way for formaldehyde content of 175 ppm and 225 ppm. the value of accuracy and precision was calculated.

### Analysis of Samples

For the analysis of samples out of the market Ciputat used the same method to measure out the simulation.

## 3. RESULTS AND DISCUSSION

Validation methods and determination of formaldehyde conducted using UV-Vis spectrophotometric method with the addition of Nash reagent. UV-Vis spectrophotometric method is a simple method. It can be used for determination of small concentrations. In addition, this method has its good sensitivity in the analysis process.

The maximum wavelength of solution of formaldehyd which reacted with Nash Reagent is 412.73 nm (According to literature is 412-415). It can be seen at figure 1.

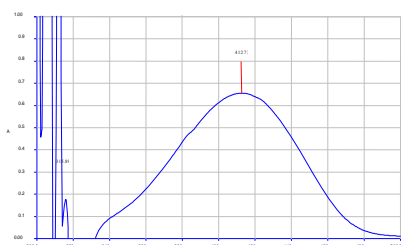
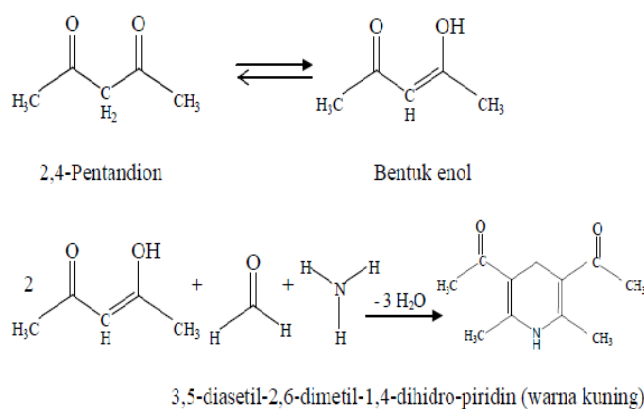


Figure 1. The maximum wavelength of solution of formaldehyd which reacted with Nash Reagent

Solution of formaldehyde is a colorless solution. The compounds that can be measured absorbance with UV-Vis spectrophotometer is an organic compound that have a chromophore group and have a color. At the sample measurement process, solution of formaldehyd is reacted with a reagent that could provide absorption spectrum of formaldehyd that is colored with Nash reagent. Nash reagent contains ammonium acetate, glacial acetic acid and acetyl acetone. When solution of formaldehyd is reacted by Nash Reagent, it produces a steady yellow color (Jon, 1980; Nash, 1953).



Sample extraction process is carried out by steam distillation. Steam distillation method performed because formaldehyde is a gaseous compound and is highly volatile or volatile. It has a boiling point 96° C. Steam distillation is required to maintain the formaldehyde compound from being damaged, because the steam distillation is used to extract the compounds that do not withstand heat or high temperatures. Formaldehyde contained in the Tofu will be bound to proteins in the tofu. The addition of phosphate acid is intended to destroy or release the bond between formaldehyde with proteins so the formaldehyde can be separated by steam distillation process. The samples were prepared directly extracted using steam distillation temperature of 96 ° C. Volumetric flask filled with aquadest 10 ml, and then submerged into the water cooler end. It aims to capture the formaldehyde vapor generated into the water distillation process that has been

accommodated. After obtaining distillate 100 ml, distillation process was stopped.

The objective of validation of an analytical method is to demonstrate that the procedure, when correctly applied, produces results that are fit for purpose. Validation of analytical method begins by making the calibration curve and determination of linearity. Calibration curve is the relationship between the absorbance value of the analyte to the concentration of the analyte. The linearity is the ability of analytical procedure to produce test results which are proportional to the concentration (amount) of analyte in samples within a given concentration range, either directly or by means of a well-defined mathematical transformation. The value of correlation coefficient (r) is good if it is 0.999. This means increasing the value of the analyte absorbance is directly proportional and significantly with increasing concentration. calibration curve generated with the equation  $Y = 0.0032 x - 0.0079$  and correlation coefficient (r) = 0.9992. Formaldehyde calibration curve in a series of concentration (ppm) against absorbance can be seen in Figure 2.

Limit of Detection (LOD) is the lowest analyte concentration in the samples can still be detected (Gholib, 2006). The experimental results obtained LOD value of 11.1328 µg / ml. Limit of quantitation (LOQ) is the smallest quantity of analyte in the samples can still be determined by the method used and meet the criteria carefully and thoroughly. The LOQ values obtained of 37.1094 g / ml. The result of the calculation of LOD and LOQ can be seen in table 1.

Table 1. The result of the calculation of LOD and LOQ

$\sum (y - y)^2$	0,00042294
$S(y/x)^2$	0,00014098
$S(y/x)$	0,011875
LOD (Limit Of Detection)	11,1328 µg/ml
LOQ (Limit Of Quantitation)	37,1094 µg/ml

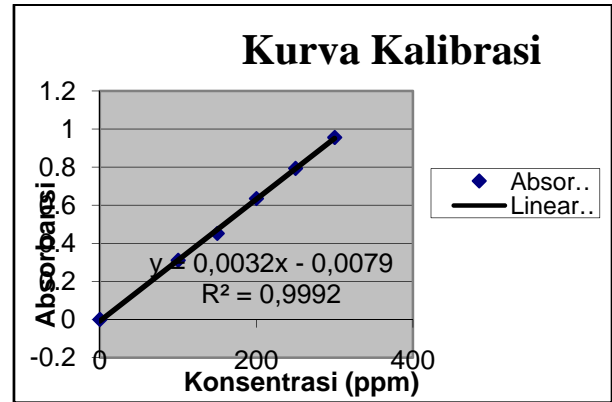


Figure 2. Formaldehyde calibration curve in a series of concentration (ppm) against absorbance

Accuracy is the closeness of the results obtained by determination of actual results and expressed in percent recovery. Percent recovery of three different concentrations of formaldehyde are 99.14% for the concentration of 125 µg/ml, 98.59% for the concentration to 175 µg/ml and 98.36 % for the concentration of 225 µg/ml. The average of percent recovery is 98.69% with standard deviation (SD) of 0.4086. Recovery test requirement is 98-102%, so the results of this test meets the requirements. Results of Recovery test of formaldehyde in tofu simulation can be seen in table 2.

Table 2. Results of Recovery test of formaldehyde in tofu simulation.

Concentration (µg/ml)	Percent of Recovery	Average ±SD (%)
125	99,14	98,69 ± 0,4085
175	98,59	
225	98,36	

Results of precision test of formaldehyde in the tofu simulation can be seen in table 3.

Table 3. Results of precision test of formaldehyde in the tofu simulation

C (actual) ( $\mu\text{g/ml}$ )	Means UPK (%)	(x - x)	((x - x) <sup>2</sup> )	SD	KV
125	99,14	0,45	0,2025	0,4085	0,4139%
175	98,54	- 0,15	0,0225		
225	98,36	- 0,33	0,1089		
X = 98,69		$\Sigma$	0,3339		

The standard deviation (SD) of data obtained at 0.4086 and coefficient of variation (CV) of 0.4139%. Coefficient of variation requirement is <2%, so the results of this test meets the requirements. From these parameters, the method could be used for samples analysis obtained from traditional market Ciputat.

Results of Determination concentration formaldehyd from traditional market Ciputat can be seen in Table 5.

Table 5. Results of Determination of formaldehyd from traditional market Ciputat.

Sample	Concentration( $\mu\text{g/ml}$ )
1	104.87
2	11.21
3	1.96
4	190.80
5	201.98
6	10.47
7	3.31

#### 4. SUMMARY

1. The validation method of determination of formaldehyde in the Tofu with the colorimetric method can be done.

2. Results of sample analysis from Ciputat market, some samples were detected containing formaldehyde. Formaldehyde concentrations are 104,87  $\mu\text{g/ml}$ , 11,21  $\mu\text{g/ml}$ , 1,96  $\mu\text{g/ml}$ , 190,80  $\mu\text{g/ml}$ , 201,98  $\mu\text{g/ml}$ , 10,47  $\mu\text{g/ml}$ , and 3,31  $\mu\text{g/ml}$ .

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