Identification of Formalin in Raw Tofu from Sidenreng Rappang District Traditional Markets

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ABSTRACT: Formalin (Formaldehyde) is a chemical compound whose use is prohibited as a preservative in food additives. This study aims to analyze the content of formalin (Formaldehyde) in raw tofu circulating in Sidenreng Rappang district. The test method used was by qualitative analysis using Schiff reagent and KMnO₄. The quantitative analysis using the UV-Vis spectrophotometry method. The test results used Schiff reagent and KMnO₄, the four tofu samples contained formalin. Test results on the UV-Vis Spectrophotometry method at a wavelength of 555 nm, obtained formalin levels in sample A = 1.130%, sample code B = 1.826%, sample code C = 1.84%. It can be concluded that in the formaldehyde qualitative analysis test on raw tofu, the three samples positively contained formaldehyde. As well as the results of the measurement of the three positive samples containing formaldehyde using a UV-Vis spectrophotometer

KEYWORDS: Formalin; Raw Tofu; Schiff and KMnO₄; UV-Vis Spectrophotometry.

1. INTRODUCTION

The distribution of food products in society needs attention in terms of safety. Several studies have been carried out to ensure the safety of food products on the market (Fawwaz et al., 2022). Tofu is a soft solid food product made from soybeans through protein precipitation and without the addition of other permitted ingredients. The chemical composition of tofu varies depending on the type of soybean used. The general composition of tofu is as follows: water content 84-90%, protein 5-8%, fat 3-4%, carbohydrates 2-4% (Indriati, 2014).

Formalin is one of the preservatives with good preservative effect. However, formalin is a chemical that is prohibited from being used in food (Daelis, 2023). According to RI Minister of Health No. 033 of 2012 concerning Amendments to Food Additives, 1168/Menkes/Per/X/1999 and Regulation of the Minister of Health Number 722/Menkes/Per/1988, that one of the prohibited food additives for use is Formalin (Formaldehyde) (Permenkes., 2012).

The use of formalin causes poisoning in the human body, the diseases that will be caused are silent diseases, namely diseases whose effects will be felt for a long time, some of which are cancer, memory loss, insomnia, fever, depression, kidney damage, decreased appetite, digestive disorders, stupidity, inflammation of the skin, anemia, convulsions, fainting, coma can even cause death (Kiroh et al., 2019).

According to research by the Institute for Science and Technology Studies (ISTECS), 90% of tofu circulating in the Bogor and South Jakarta areas uses formaldehyde as a preservative. Based on the test results for the formaldehyde content in tofu conducted at the Padang Industrial Research and Standardization Center, it was found that the formalin level was 17,100 ppm (Sari et al., 2014). Determination of formalin levels can be carried out using the fluorimetric method, HPLC, gas chromatography, and spectrophotometry. The method that is usually used is the spectrophotometric method because it is cheap and easy to use (Santoso, 2020). Therefore, it is necessary to make efforts to detect the presence or absence of formalin in food, especially tofu circulating in the community.

2. EXPERIMENTAL SECTION

2.1. Population and Sample

The population in this study was raw tofu circulating in the Sidenreng Rappang district. The sample used was tofu obtained from the Rappang Central Market, Sidenreng Rappang Regency.

2.2. Materials and tools

The materials used in this study were aquadest aluminum foil, 37% formalin, reagent 0.1 N KMnO₄, Schiff reagent, and raw tofu. The tools used were a porcelain cup centrifuge, 500 mL (pyrex®) beaker, 10 mL (pyrex®) volumetric flask and 100 mL mortar, cuvette, dropper pipette, spatula, UV-Vis spectrophotometer, test tube rack, centrifuge tube, analytical balance (Ohaous®), test tube (iwaki®).
2.3. Qualitative Analysis

2.3.1. Sample preparation

Samples of raw tofu are weighed as much as 15 g, put into a mortar then crushed until smooth, then take enough samples to soak using hot water, put it in a centrifuged tube and then centrifuged for 5 minutes at a speed of 3000 rpm using a centrifuge, the supernatant from the centrifugation was carefully pipetted into a test tube.

2.3.2. Schiff's reagent test

Samples that have been refined are taken to taste and then put into a test tube, then add 3 drops of Schiff's reagent. Observe the color change that occurs, if there is a clear color change to purple then the result is positive the sample contains formalin.

2.3.3. KMnO₄ reagent test

The sample that has been refined is then put into the reagent tube, then added 0.1 N KMnO₄ as much as 3 drops. Observe the color change that occurs, if there is a color change from purple to brown, the result is positive for the sample containing formalin.

2.4. Quantitative Analysis

2.4.1. Preparation of stock solutions for standard formalin

From a 37% formaldehyde solution, use as much as 5 mL and dilute it with distilled water up to 10 mL to obtain a concentration of 500 ppm.

2.4.2. Maximum wavelength determination

A standard solution of 37% formalin with a concentration of 500 ppm is diluted to 45 ppm by means of a pipette of 0.45 mL standard solution, then put into a 5 mL volumetric flask then added with distilled water up to the mark limit, then from a 45 ppm solution, pipette 1 mL then 0.5 mL of Schiff reagent was added, then added with 2 mL of distilled water, then homogenized and incubated for 30 minutes. Then measured at a wavelength of 400-800 nm, using UV-Vis spectrophotometry.

2.4.3. Standard curve creation

Standard 37% formalin solution, the concentration of 500 ppm that has been made, is diluted into 5 series of concentrations, namely concentrations of 100, 150, 200, 250, and 300, then from each series of these concentrations in a 1 mL pipette then added with Schiff reagent as much as 0.5 mL then add 2 mL of distilled water then homogenize and incubate for 30 minutes. The absorbance of the standard solution and blank solution was measured at a wavelength of 555 nm. Then the regression curve is measured from the absorption of the standard solution, a straight line is drawn connecting the concentration with the absorbance (absorption) and an equation y = bx + a is obtained.

2.4.4. Sample preparation and measurement

Raw tofu samples were weighed as much as 15 g, crushed first until smooth, then soaked with 30 mL of distilled water that had been put in an Erlenmeyer, shaken gently and let stand again for 5 minutes. strain the tofu mixture with filter paper. The filtrate was put into Erlenmeyer and covered with aluminum foil. The results of the tofu sample filtrate were taken 5 mL put into a 10 mL volumetric flask to obtain a concentration of 250,000 ppm, then from a concentration of 250,000 ppm, pipette 1 mL into a 10 mL volumetric flask then sufficient with distilled water until the limit mark obtained a concentration of 25,000 ppm, then from a concentration of 25,000 ppm was diluted to a concentration of 10,000 ppm, from a concentration of 10,000 ppm in a 1 mL sample pipette then added 0.5 mL of Schiff reagent then added 2 mL of aquadest then incubated for 30 minutes, and replicated 3 times for the three samples. The absorbance is measured by the maximum wavelength.

2.5. Data analysis

Data analysis was carried out by calculating the formaldehyde content in raw tofu using the sample content equation formula (Lambert Beer):

\[
\text{% rate} = \frac{C_{\text{sample}}(\text{ppm}) \times \text{sample volume(L)} \times \text{Fp}}{\text{sample weight}(\text{mg})} \times 100\%
\]

3. RESULTS AND DISCUSSION

Qualitative analysis using Schiff's reagent was carried out in a way raw tofu samples were weighed as much as 15 g, put into a mortar and then crushed until smooth, then taken sufficient samples soaked in hot water, put into a...
centrifugal tube after that centrifuged for 5 minutes at a speed of 3000 rpm using a centrifugation tool. Carefully then put it in a test tube, additional 3 drops of Schiff reagent. A positive result if there is a color change from a clear color to a purple color, where these three positive samples contain formalin because there is a color change from a clear color to a purple color. As shown in Table 1.

Table 1. The results of qualitative testing using Schiff reagent

<table>
<thead>
<tr>
<th>Sample</th>
<th>Positive Result (References)</th>
<th>Test result</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>Purple (Sari et al., 2021)</td>
<td>Purple</td>
<td>+</td>
</tr>
<tr>
<td>Sample B</td>
<td>Purple</td>
<td>Purple</td>
<td>+</td>
</tr>
<tr>
<td>Sample C</td>
<td>Purple</td>
<td>Purple</td>
<td>+</td>
</tr>
</tbody>
</table>

Qualitative analysis was carried out using Schiff reagent and reagent KMnO₄, Schiff's reagent is a sensitive and selective reagent in detecting Formaldehyde up to a concentration of 0.01 ppm. Schiff reagent and formalin react to produce a purple color which is the result of a condensation reaction between formalin (formaldehyde) which contains a carbonyl group (C=O) and Schiff’s solution (Pratiwi, et al., 2019).

Qualitative analysis using KMnO₄ reagent was carried out by weighing 15 g of raw tofu sample, putting it in a mortar, then grinding it until smooth, then taking an adequate sample, soaking it in hot water, putting it in a centrifugal tube after that, centrifuging it for 5 minutes at 3000 rpm using a tool. Centrifugation. Pipette the supernatant from centrifugation carefully then put it into a test tube, add 3 drops of KMnO₄ reagent, then homogenize and let stand for 5 minutes, sample A changes color to red-purple, for both samples B and C change color to brown color, this happens because sample A has a slightly different formaldehyde content from samples B and C which have a lot of formalin content, then allowed to stand again for 30 minutes a color change occurred for sample A from purplish-red to brown and gradually changed color to a clear color. Meanwhile for samples B and C after being left for 30 minutes the brown sample gradually faded to a clear color.

The qualitative test results for the KMnO₄ reagent in the three samples showed a positive test result which was indicated by a change from purple to brown. KMnO₄ and formalin can react to produce a brown color based on an oxidation reaction (Pratiwi, et al., 2019). the results of qualitative analysis testing can be seen in Table 2.

Table 2. The results of qualitative testing using KMnO₄ reagent

<table>
<thead>
<tr>
<th>Sample</th>
<th>Positive Result (Pratiwi et al.)</th>
<th>Results Testing (5 minutes)</th>
<th>Test result (30 minutes)</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>Brown – clear</td>
<td>Purplish red</td>
<td>Clear</td>
<td>+</td>
</tr>
<tr>
<td>Sample B</td>
<td>Brown – clear</td>
<td>Chocolate</td>
<td>Clear</td>
<td>+</td>
</tr>
<tr>
<td>Sample C</td>
<td>Brown-clear</td>
<td>Chocolate</td>
<td>Clear</td>
<td>+</td>
</tr>
</tbody>
</table>

From the qualitative analysis it can be seen that the sample tested was raw tofu which contained formalin. To ensure further, a quantitative analysis was carried out using a UV-Vis Spectrophotometer.

The maximum wavelength obtained was 555 nm, where the purpose of determining the maximum wavelength is to determine the maximum absorption of formaldehyde. The wavelength obtained will be used in measuring the absorbance of the sample (Rahmadari, et al., 2021). Standard curve solutions are made with several concentrations as shown in Table 3.

Table 3. Standard curve measurement results

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0.230</td>
</tr>
<tr>
<td>150</td>
<td>0.329</td>
</tr>
<tr>
<td>200</td>
<td>0.488</td>
</tr>
<tr>
<td>250</td>
<td>0.640</td>
</tr>
<tr>
<td>300</td>
<td>0.720</td>
</tr>
</tbody>
</table>
After measuring the absorbance, a graph of the formaldehyde standard curve was then made to obtain a linear regression equation. The formalin (formaldehyde) standard curve graph can be seen in Figure 1.

![Linear regression graph](image)

**Figure 1. Linear regression**

Based on the formaldehyde standard curve graph, a linear regression equation of the standard curve is $y = 0.0026x - 0.035$ with a correlation coefficient ($r$) of 0.994. Where 0.0026x is the value of b (slope), 0.035 is the value of a (intercept). If the value of $r$ is less than 0.999 then the parameter $V_{x0}$ must be calculated. $V_{x0}$ is a parameter to determine the linearity of a method, by measuring the closeness of the area measured by the detector to the area obtained from the calculation of the standard curve equation. Where the test is said to meet the requirements if $V_{x0} < 5\%$, then from the linear regression equation the calibration curve with the linear regression equation ($y = 0.0026x - 0.035$) and $V_{x0} 4.915\%$ can be used to determine the level of formalin in raw tofu.

In determining the level of formalin samples of raw tofu was carried out with 3 replications on samples ab and c with the same treatment, where replication is the number of experimental units that receive the same treatment under certain conditions (Zainuddin, 2014). The goal is to do 3 replications to increase the accuracy of the absorbance value in research or reduce the error rate of a study so as to get accurate absorbance values (Munriyastutik et al., 2020; Fawwaz et al., 2023). Based on the calculation results in Table 4, the sample coded sample a had a lower % content compared to the other two samples, where the average % formality content was 1.13%. This can be seen from the results of the qualitative analysis, where the sample coded sample a produces a color that is not as intense when compared to other samples. While the average % content for samples coded sample b, sample c, respectively 1.83%, 1.85%. The results of calculating the percent content in the sample can be seen in Table 4.

<table>
<thead>
<tr>
<th>No</th>
<th>Sample Code</th>
<th>Repetition</th>
<th>Absorbance</th>
<th>Content (%)</th>
<th>Average (%) content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample A</td>
<td>1</td>
<td>0.245</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0.280</td>
<td>1.21</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>0.254</td>
<td>1.11</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sample B</td>
<td>1</td>
<td>0.466</td>
<td>1.92</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0.450</td>
<td>1.86</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>0.409</td>
<td>1.70</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Sample C</td>
<td>1</td>
<td>0.455</td>
<td>1.88</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0.405</td>
<td>1.69</td>
<td>1.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>0.475</td>
<td>1.96</td>
<td></td>
</tr>
</tbody>
</table>

From the results obtained, based on the Regulation of the Minister of Health of the Republic of Indonesia No. 033 of 2012 concerning Amendments to Food Additives, 1168/Menkes/Per/X/1999 and Regulation of the Minister of Health Number 722/Menkes/Per/1988, that one of the prohibited food additives for use is Formalin (Formaldehyde) (Permenkes, 2012). Therefore, the three samples tested in this study should not be consumed and not traded to the public because they can endanger the health of the body.

**4. CONCLUSION**

Based on the research results obtained, it can be concluded that in the formaldehyde qualitative analysis test on raw tofu, the three samples positively contained formaldehyde. As well as the results of the measurement of the three positive samples containing formaldehyde using a UV-Vis spectrophotometer, the % value of formalin levels was obtained for sample A, sample B, sample C, respectively 1.13%, 1.83%, 1.85%.
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REFERENCES


Zainuddin, M. Metodologi Penelitian Kefarmasian dan kesehatan edisi 2, Surabaya, AUP. 2014.

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