Analysis of Rhodamine B Content in “Geplak” Marketed in Yogyakarta City in 2019

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Abstract. Rhodamine B is a coloring agent which is prohibited to add in food. Rhodamine B often used to color food product, beverage, and cosmetics. Rhodamine B is a synthetic coloring agent used in the textile and paper industry, this synthetic coloring agent is dangerous when inhaled, affects the eyes and skin, and is swallowed. The purpose of this study was to identify and know the levels of rhodamine B in geplak samples circulating in the city of Yogyakarta. Geplak samples studied amounted to 5 samples taken from souvenir shops in the city of Yogyakarta. The method used to identify rhodamine B in geplak was soaking the samples in ammonia to pull out rhodamine B dyes using wool yarn, followed by identification using thin layer chromatography with the mobile phase of n-butanol: ethyl acetate: ammonia (10: 4: 5). Then it was detected with UV light of 254 nm and 366 nm. The analysis of rhodamine B levels and the maximum wavelength of the sample was carried out using UV-Visible spectrophotometry. The results showed that the 5 samples examined by thin layer chromatography or UV-Visible spectrophotometry were not identified as containing rhodamine B.

1. Introduction
Food safety is an important requirement that must be obeyed in the food that will be consumed by everyone [1]. Foods that have good taste, practical, and instant can contain additives that contain chemicals that are toxic. The ingredients added to the food are called food additives (BTP).

One type of food product that often uses additives in the form of coloring agents is geplak which is one of the typical food of Yogyakarta. Therefore, the increasing number of geplak production is feared by the misuse of non-food synthetic dyes such as rhodamine B [2].

Rhodamine B is one of the dyes which is declared as a hazardous material for health and is prohibited to be used as a food additive [3]. Rhodamine B is a synthetic coloring agent used in the textile and paper industry, this synthetic coloring agent is very dangerous when inhaled, affects the eyes and skin, and is swallowed. In more acute conditions can interfere with liver function and cause liver cancer [4].

Many studies have proven the abuse of rhodamine B added as a coloring agent in food products, it was found that some food products that were positive red contained rhodamine B [5, 6]. Based on this, it is necessary to conduct research on the content of rhodamine B in geplak marketed in Yogyakarta City. Analysis of synthetic dyes on food and beverages can be done both qualitatively and quantitatively using thin layer chromatography and UV-Visible spectrophotometry.
2. Experimental
2.1. Materials
Rhodamine B (Merck), ammonia (Merck), glacial acetic acid (Merck), n-butanol (Merck), ethyl acetate (Merck), ethanol (Merck), aquadest (Agung Jaya), wool yarn, 5 red geplak samples marketed in Yogyakarta, analytical balance (Sartorius BP110), hot plate, UV-Visible spectrophotometer (Shimadzu 1601PC).

2.2. Preparation Sample Method
A total of 10 g of sample geplak carefully weighed. The sample was extracted by soaked in 10 mL of 2% ammonia solution (in 70% ethanol) for 24 hours, then the solution was filtered. The results of the solution were then evaporated on a hot plate. The residue from heating was dissolved in 6 mL of acidic water (water: 10% acetic acid 2: 1). A 15 cm long wool yarn was put into the acid solution and boiled for 20 minutes until the dye was absorbed in the wool yarn and coloring the wool yarn. Wool yarn was removed and washed with 2 mL of water. The wool yarn was then put into 10 mL of 10% ammonia solution (in 70% ethanol) and boiled. Wool yarn will release the dye into base solution. The obtained base solution was then concentrated. The results of this extraction can be used as samples in qualitative tests with thin layer chromatography [7]. Qualitative test was done by UV-Vis spectrophotometry, using the samples from the results of the extraction which were added with 5 mL of water.

2.3. Qualitative Analysis Using Thin Layer Chromatography (TLC)
Qualitative analysis of rhodamine B in geplak was carried out by thin layer chromatography (TLC) method. Samples and standard solution of rhodamine B were spotted in silica gel GF254 stationary phase and developed with the mobile phase of n-butanol: ethyl acetate: ammonia (10: 4: 5). Visually stained pink and under UV lamps 254 and 366 nm yellow or orange fluorescence and the Rf value of the sample was the same as the Rf value of the standard rhodamine B solution, this indicates the presence of rhodamine B content [8].

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Rf = \frac{\text{distance traveled by the center of a spot}}{\text{distance traveled by the solvent front}}
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2.4. Analysis using Spectrophotometer UV/Vis
2.4.1. Scanning wavelength rhodamine B standard
Rhodamine B solution with a concentration of 2.25 μg/mL was read its absorbancy at 400-800 nm wavelength with a UV-Vis spectrophotometer.

2.4.2. Standard Curve of Rhodamine B
Rhodamine B standard curve was obtained from dilution of 1000 μg/mL standard solution to 1.5 series concentration solution; 1.75; 2; 2.25; 2.5; 2.75; and 3 μg/mL. The concentration series solution was read its absorbance at maximum wavelength. The absorbance result data can be calculated the standard curve equation so that the linear equation \( y = bx \pm a \) was obtained.

2.4.3. Scanning wavelength sample for qualitative analysis
The results of the preparation of rhodamine B in samples that have been added with 5 mL of water were then read at wavelengths of 400-800 nm.

2.5. Data Analysis
The results of qualitative analysis with TLC seen by comparing the value of Rf sample Rf to the standard Rf value of rhodamine B. Furthermore, the results of qualitative analysis with UV/Vis spectrophotometer were obtained by comparing the maximum wavelength between the sample with the standard rhodamine B. If the results of qualitative analysis showed positive results then continued
by quantitative analysis using a spectrophotometer by looking at the absorbance value of the sample and entering it into the standard curve equation, so rhodamine B levels in the sample were obtained.

3. Results and Discussions

3.1. Qualitative Analysis of Sample Geplak Using TLC

The five samples identified by TLC showed that they were not containing rhodamine B dye. The Rf value of the sample was compared with the comparison standard rhodamine B. Samples A, B, C, D and E did not produce spots so the Rf value could not be calculated. The result of Rf is positive if the color of the spots between the sample and the standard of comparison are the same and the value of Rf between the sample with the standard of comparison are equal or close to each other with the difference ≤ 0.2 [9]. The results of qualitative analysis by TLC can be seen completely in Table 1.

3.2. Qualitative Analysis of Sample Geplak Using Spectrophotometer UV/Vis

Qualitative analysis can also be carried out using a UV/Vis spectrophotometer. Rhodamine B qualitative analysis uses a UV/Vis spectrophotometer by comparing the absorbance spectra at a wavelength of 400-800 nm. The maximum wavelength of rhodamine B solution was 554 nm. The maximum wavelength obtained was in accordance with the literature using water solvents that is 554 nm [10, 11].

The results of determining the maximum wavelength in the sample are not found any samples that have the same maximum wavelength or close to the maximum wavelength of standard rhodamine B, so it can be concluded that all samples were not containing rhodamine B. The results of qualitative analysis by UV/Vis spectrophotometer can seen completely in Table 2.

![Figure 1. TLC Profile of Rhodamine B Standard, Sample+Rhodamine B, and Sample Under: UV lamp at 254 nm (A), UV lamp at 366 nm (B), and visual (C)](image)

| Sample                  | Visual | Under UV Detection | Rf | Result |
|-------------------------|--------|--------------------|----|--------|
|                         |        | 254 nm             | 366 nm |        |
| Standard                | Pink   | Orange             | Orange | 0.66   |
| Standard + sample C     | Pink   | Orange             | Orange | 0.6    |
| Sample A                | -      | -                  | -    | (-)    |
| Sample B                | -      | -                  | -    | (-)    |
| Sample C                | -      | -                  | -    | (-)    |
| Sample D                | -      | -                  | -    | (-)    |
| Sample E                | -      | -                  | -    | (-)    |
Table 2. Maximum Wavelength of Samples and Standard rhodamine B

| Sample | Wavelength |
|--------|------------|
| Standard | 554 nm |
| A       | 528 nm |
| B       | 528 nm |
| C       | 508 nm |
| D       | 527 nm |
| E       | 528.5 nm |

Figure 2. Maximum Wavelength of Rhodamine B

4. Conclusion
The results of the analysis of 5 geplak samples by thin layer chromatography and UV/Vis spectrophotometer were not identified as containing rhodamine B.

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