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Research Article

Analysis of Rhodamine B Levels in Cotton Candy Circulated in Makassar City

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ABSTRACT: Rhodamine B is a synthetic dye used as a textile dye. It is prohibited as a Food Additive because it can irritate the digestive tract, poison, and even cause cancer and death. The research was to see if there were any Rhodamine B deposits of cotton candy in Makassar. This test used five samples. The methods used in testing are TLC and spectrophotometry UV-Vis. All five samples' test results show no stain was formed (negative). UV-Vis spectrophotometry test on a 553 nm length acquired data of Rhodamine B levels in a row as follows: first sample of cotton candy = 0.005%, sample 2 of cotton candy = 0.001%, sample 3 of cotton candy = 0.0008%, sample 4 of cotton candy = 0.003%, and sample 5 of cotton candy = 0.0009%. They concluded that all five samples identified contained Rhodamine B synthesized dye to a minimal degree of the cotton candy sample distributed in Makassar.

KEYWORDS: Cotton candy; Rhodamine B; TLC; UV-Vis spectrophotometry.

1. INTRODUCTION

Cotton candy is a traditional Indonesian food that was popular at the time. When there weren't as many packaged candies or lollipops as now, children preferred buying cotton candy sold by itinerant vendors. Sweet arum is made from the sugar added with food coloring (Kusumaningrum and Fitriah, 2020). However, currently, children's snacks are a problem that needs to be considered by the community, especially parents and teachers, because they have a very high risk of biological and chemical contaminants that can interfere with health, both in the short term and in the long term (Tjiptaningdyah, 2016).

Rhodamine B is a coloring substance that is prohibited from being used according to PERMENKES RI No.239/Menkes/Per/V/1985 it is a type of dye that is toxic if given to food or drink; it can hurt human health. The liver cannot metabolize Rhodamine B, so it builds up in the liver, which will cause liver dysfunction. The chemical structure of Rhodamine B contains the element N+ (nitronium), which is carcinogenic, thus spurring the growth of cancer cells and causing cancer and liver tumors (Hadriyati et al., 2021).

The characteristics of food containing Rhodamine B include bright, shiny, and more conspicuous colors; sometimes, the color looks uneven, there are clumps of color in the product, and the taste is slightly bitter when consumed. Usually, food products containing this substance do not include a code, label, brand, or other complete identity (Adriani et al., 2019).

Products suspected of containing Rhodamine B can be analyzed qualitatively and quantitatively. For qualitatively using thin layer chromatography (TLC), wool method, test kits, and staining tests. While quantitative by using a UV-Vis Spectrophotometer to determine the levels of Rhodamine B in food (Saputri, 2018).

2. EXPERIMENTAL SECTION

2.1. Population and Sample

The population in this study is Cotton candy circulating in Makassar which does not have a production permit from BPOM. The sample used in this study was cotton candy snacks that did not have a production permit from BPOM, consisting of 5 samples taken randomly from several subdistricts, namely 2 samples from the Bontoala subdistrict, 1 sample from Biringkanaya subdistrict, 1 sample from the Tamalanrea subdistrict, and 1 sample from Ujung Pandang.

2.2. Tools and Materials

Stirring rod, wool yarn, chamber (Camag®), 50 mL separating funnel (Pyrex®), 50 mL and 100 mL beaker (Pyrex®), 10 mL and 100 mL measuring cylinder (Pyrex®), hot plate (Cimarec®), cuvette, 5 and 10 mL volumetric flask (Iwaki®), 100-1000 µL micropipette (DragonLAB), pipette, TLC plate (Silica Gel 254 10 x 10), 254 nm UV light, UV-Vis spectrophotometer (Thermo Scientific), analytical balance (Kern®), vials, concentrated ammonia, 0.1 N hydrochloric acid, glacial acetic acid, distilled water, Rhodamine B standard, diethyl ether, n-butanol, 10% sodium hydroxide and Cotton candy.

2.3. Preparation of Wool Yarn and Samples

2.3.1. Degreasing of Wool Yarn

Cut the wool thread 30 cm long, then boil the wool thread with 50 mL of distilled water in a chemical glass, then dry it using a tissue, wash it using chloroform solution, boil it again with 1% sodium hydroxide solution, then rinse it using distilled water, and dry it again by using a tissue.

2.3.2. Sample Preparation

The sample was weighed as much as 3 g, dissolved each sample in 40 mL of distilled water, then added 10 drops of 10% acetic acid, put the wool thread, and heated over a water bath while stirring until the color adhered to the wool thread was removed, then the colored wool thread was washed repeatedly until clean with distilled water. Wool yarn that has been cleaned is put into a beaker, added 10 mL of distilled water and 5 drops of concentrated ammonia, and heated over a water bath until the remaining solution in the beaker is approximately 1 mL. The thread in the beaker is squeezed using a stir bar. The juice is collected in the vial and ready for use as a TLC and UV-Vis Spectrophotometer sample.

2.4. Qualitative Analysis

2.4.1. Staining Test

As much as 2 g of sample was put into a 50 mL beaker glass, then dissolved with 30 mL of hot distilled water, stirring until dissolved, then left to cool. Take 3 mL of the test solution and add 10% NaOH drop by drop until it becomes alkaline, then put it into a separatory funnel, then add 3 mL of diethyl ether and shake and separate to take the ether phase. Then add 1 mL of 0.1N HCl. If the test solution contains Rhodamine B, it will appear on the red bottom layer (Taupik, 2021).

2.4.2. Thin Layer Chromatography (TLC)

Prepare the Rhodamine B standard solution by weighing 5 mg of Rhodamine B standard and dissolving it with 10 mL of distilled water. A pipette of as much as 400 µL dissolved in 10 mL of distilled water.

Spot the sample and reference on the TLC plate, insert the TLC plate into the chamber which has been saturated with the eluent (n-butanol: glacial acetic acid: distilled water, 4: 4: 4). Then it was left for a while until the eluent rose and touched the plate boundary. Then the plate was removed and dried with a dryer. The TLC plate was placed under a UV lamp with a wavelength of 254 nm and 366 nm, and then the spots were marked. From the spots obtained, the Retention factor value can be calculated (Duhita, 2020).

2.5. Quantitative Analysis

2.5.1. Rhodamine B Standard Preparation

The Rhodamine B standard solution was prepared by weighing 5 mg of Rhodamine B standard and then dissolving it with 10 mL of distilled water, pipetted as much as 0.4 mL, and dissolved in 10 mL of distilled water to make a standard solution with a concentration of 20 ppm. The standard solution with a concentration of 20 ppm was put into the cuvette and then measured at a 400-800 nm wavelength with distilled water as a blank.

Standard solution with a concentration of 20 ppm was made into several series of concentrations, namely 1.5; 2; 2.5; 3; 3.5; and 4 ppm. Then it is measured at the maximum wavelength obtained in the previous wavelength measurement, with distilled water as a blank.

2.5.2. Determination of Rhodamine B by UV-Vis Spectrophotometric

The UV-Vis spectrophotometric method was performed following the previous study with modification (Fawwaz et al., 2017). Each sample that has been prepared is then put into the cuvette. The Rhodamine B levels in the sample were calculated using a visible light spectrophotometer at the maximum wavelength.

2.6. Data Analysis

Data analysis can be done by calculating the levels of Rhodamine B in Cotton candy using the formula for the sample content equation (Lambert-Beer):

$$\% \text{ content} = \frac{\text{C sample (ppm) x Sample Volume (L) x Fp}}{\text{Sample Weight (mg)}} \text{ x } 100\%$$

3. RESULTS AND DISCUSSION

The initial stage carried out in this study was qualitative analysis using the Color Test. The material used is hot distilled water to dissolve the cotton candy samples. Hot distilled water separates the dyes in the sample because if you use distilled water at normal temperature, the solubility of the dyes in the sample will decrease. The next ingredient is the addition of sufficient NaOH until the pH solution is alkaline and then given diethyl ether. This is so that Diethyl Ether can easily withdraw the Rhodamine B dye dissolved in the alkaline NaOH environment because Rhodamine B is a group of alkaline dyes, therefore when it is in a non-ionized state or at a high pH, basic compounds tend to dissolve well in solvents. The last ingredient is HCl, the addition of HCl is intended so that the Rhodamine B dye can be withdrawn from the ether phase into an acidic environment to obtain color identification results (Taupik, 2021). Positive indicates whether the bottom or acid layer is red as shown in **Table 1**.

Table 1. Qualitative Identification of Rhodamine B by staining test

Sample	Positive Result (Reference)	Test Results	Information
Cotton Candy S1	Red	Clear	-
Cotton Candy S2	Red	Clear	-
Cotton Candy S3	Red	Clear	-
Cotton Candy S4	Red	Clear	-
Cotton Candy S5	Red	Clear	-

The results of qualitative analysis in **Table 1** using the staining test were obtained for samples S1, S2, S3, S4, and S5. The colors produced were clear or negative (-) containing Rhodamine B. Next is a qualitative analysis using TLC. The parameter used as the basis for identification in TLC is the Rf value, where a compound is declared identical if it has the same Retention factor value. The results of the identification of Rhodamine B dye in cotton candy using the TLC method in the standard or reference solution of Rhodamine B produces a visually pink stain color when viewed under UV light 254 and 366 nm it has yellow and orange fluorescence (Muzdhalifah, 2019) with a spot height of 6.5 cm (**Table 2**). The five samples of cotton candy did not show the same spots as standard Rhodamine B spots and did not show any stains formed on the TLC plate. Qualitative and quantitative analysis was carried out using a UV-Vis Spectrophotometer to ensure further the presence or absence of Rhodamine B in cotton candy.

Table 2. Identification of Rhodamine B in TLC

Sample	Shooting Volume	Spot Height	Rf Value	Spot Color	Results
Sample S1	2 µL	-	-	-	-
Sample S2	2 µL	-	-	-	-
Sample S3	2 µL	-	-	-	-
Sample S4	2 µL	-	-	-	-
Sample S5	2 µL	-	-	-	-
Comparison (B)	2 µL	6.5	0.812	Pink	

Determination of the maximum wavelength aims to determine the optimum wave absorption area of Rhodamine B, which will then be used to measure the absorbance of the sample (Anngela, 2021). The maximum wavelength obtained is 553 nm. The standard curve solution was made with several concentration variations, as shown in **Table 3**. Solutions of six concentration variations were measured at the maximum wavelength so that the absorbance value of each concentration series was obtained. A linear line equation was obtained, which could later be used to determine the levels of Rhodamine B in cotton candy samples.

Table 3. Standard curve measurement results

Concentration (ppm)	Absorbance (553 nm)
1.5	0.275
2.0	0.376
2.5	0.466
3.0	0.545
3.5	0.641
4.0	0.761

The calibration curve is a graph that forms a linear or straight line which is the relationship between the instrument's response and a certain known concentration (Sri, 2019). The line equation is obtained from the calibration curve, which states the relationship between concentration and absorbance. The regression equation was obtained from the standard curve, y = 0.1888x – 0.0085, with an R-value of 0.9982. The value of 0.1888x is the value of the slope (b), 0.0085 is the value of the intercept (a), and 0.9982 is the value of the correlation coefficient (r). The value of a good correlation coefficient is close to 1 (Fawwaz et al., 2022). According to the terms of acceptance, the correlation coefficient obtained is 0.9982.

The results of calculating the levels of Rhodamine B in 5 samples of cotton candy taken from 5 different traders, the absorbances obtained from the five samples were 0.566, 0.215, 0.091, 0.343, and 0.094, respectively (**Table 4**). Then the percentage of Rhodamine B levels was obtained from 5 samples in a row, namely 0.005%, 0.001%, 0.0008%, 0.003%, and 0.0009%. From the results obtained, based on the Regulation of the Minister of Health of the Republic of Indonesia No. 239 of 1985, one of the dyes that are prohibited from being used in a food product is Rhodamine B.

Table 4. Determination of Rhodamine B levels in cotton candy

Sample	Absorbance (553 nm)	Percentage (%)
Bontoala S1	0.566	0.005%
Bontoala S2	0.215	0.001%
Biringkanaya S3	0.091	0.0008%
Tamalanrea S4	0.343	0.003%
Ujung Pandang S5	0.094	0.0009%

From the results obtained, it was known that the five samples had Rhodamine B levels but in tiny amounts. Even so, the textile dye Rhodamine B should not be consumed according to the Regulation of the Ministry of Health of the Republic of Indonesia Number 239 of 1985 (Budimarwanti, 1992). This is because it can cause poisoning and can even result in death (Tjiptaningdyah, 2017).

4. CONCLUSION

Based on the analysis of the data obtained from the study, it can be concluded that the qualitative test results for coloring the five cotton candy samples did not contain Rhodamine B. For the TLC test results, the Rf value for the standard was 0.812. If observed visually, the stains formed are pink in color, but if observed under UV light at 254 and 366 nm, they fluoresce yellow and orange. Of the five samples, no stains were formed in the TLC test, which indicated that the five samples did not contain Rhodamine B. Meanwhile, the test results on the UV-Vis Spectrophotometer at 553 nm wavelength showed that the five samples of cotton candy were positive for containing Rhodamine B with sample levels cotton candy respectively, namely Bontoala S1 = 0.005%, Bontoala S2 = 0.001%, Biringkanaya S3 = 0.0008%, Tamalanrea S4 = 0.003%, and Ujung Pandang S5 = 0.0009%.

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