

## Analysis of rhodamine b content in beef sausages sold in the yogyakarta city market using visible spectrophotometry method

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### ABSTRACT

Rhodamine B is a synthetic dye commonly used as a textile dye. According to the Minister of Health of the Republic of Indonesia No. 033 of 2012, rhodamine B is an additional colorant prohibited from being used in food products because of its carcinogenicity. This study aimed to determine the presence of rhodamine B in beef sausages sold at the Yogyakarta City Market by visible spectrophotometry. The sample used in this study was a beef sausage with a reddish-pink characteristic and without a brand. The result analysis method used in determining the qualitative test is by comparing the sample wavelength with the wavelength of the Rhodamine B standard and using the Thin Layer Chromatography (TLC) method. In the quantitative analysis, the Visible Spectrophotometry method was used. The data obtained were then analyzed using the SPSS Kolmogorov-Smirnov method, then ANOVA was used. The results of the qualitative test study obtained three positive samples containing Rhodamine B from a total of 7 samples tested. Determination of Rhodamine B levels in samples using Visible Spectrophotometry with a maximum wavelength of 556.0 nm. Rhodamine B levels obtained in samples A, C, and G, were  $1.06 \pm 0.67$ ,  $0.87 \pm 0.81$ , and  $1.04 \pm 0.82$  ppm. Conclusions in the Kolmogorov-Smirnov test, the data were normally distributed, and the ANOVA results showed no differences in levels between samples.

**Keywords:** Sausage, Rhodamin B, TLC, UV-Vis, Validation.

### INTRODUCTION

One of the prohibited dyes is rhodamine B which is declared dangerous if found in food and beverages, which is regulated through the Decree of the Minister of Health of the Republic of Indonesia Number 239/Menkes/Per/V/85. The impacts that occur can be in the form of irritation to the respiratory tract, irritation to the skin, irritation to the eyes, irritation to the digestive tract, and dangerous for liver cancer. It can irritate the digestive tract if swallowed, and urine will be red or pink (Pertiwi et al., 2013). Consuming rhodamine B in the long term can accumulate in the body. It can cause symptoms of liver and kidney enlargement, impaired liver function, liver damage, physiological disorders of the body, or can even cause liver cancer (Hidayah et al., 2017).

The increasing population partly causes the emergence of abuse of these dyes, and the increasing human needs, resulting in tight competition in the business world. Competition in the business world is getting tougher, so many companies try to survive by looking at consumer desires for a product (Silalahi & Rahman, 2011).

According to the National Standardization Agency (SNI 01-3820-1995), sausage is a food product obtained from a mixture of finely ground meat (containing not less than 75% meat) with flour or starch with or without the addition of spices and other permitted food additives and put in sausage sleeve. An essential component of meat in the manufacture of sausages is protein. Meat protein plays a role in increasing the breakdown of meat during cooking to form a compact product structure (Badan Standardisasi Nasional, 1995)

According to SNI 01-2895-1992, the content of coloring additives in sausages can be determined using several methods, namely in qualitative analysis using the paper chromatography method using wool yarn, polyamide column, and in quantitative analysis using the TLC Scanner method (Badan Standardisasi Nasional, 1992; Purnamasari & Saebani, 2016). However, in this study, the quantitative analysis method was chosen using the Uv-Visible Spectrophotometry method (Permatasari et al., 2014; Suhartati, 2017; Zackiyah, 2016).

## RESEARCH METHOD

### Materials

The materials used in this study were 2% Ammonia, 10% Ammonia, 10% Acetic Acid (Merck), Aquadestilata, standard rhodamine B (Merck), wool yarn, ether, distilled water, 10% ammonia, 70% ethanol (Bratachem), silica gel GF 254 nm, eluent, n-Butanol (Merck): Ethyl Acetate (Merck): Ammonia (Merck) (10:4:5), Sausage Sample A, Sausage Sample B, Sausage Sample C, Sausage Sample D, Sample Sausage E, Samples of Sausage F, Samples of Sausage G (sausages were taken from seven markets in Yogyakarta City which included red sausages, not branded, using purposive sampling method).

The tools used are analytical balance, hot plate, Whatmann filter paper No. 1, UV 1800 spectrophotometry (Shimadzu), a set of thin layer chromatography tools, proportions, water bath, 366 nm UV lamp, and glassware.

### Methods

#### Sampling Technique

A sampling of beef sausages that are reddish pink and without a brand was carried out in 7 markets in the city of Yogyakarta in March - April 2021 with a total market population of 32 markets in the city of Yogyakarta selling beef sausages, then calculated by the formula  $\sqrt{n} + 1$ , where n is the population so that seven samples are taken to be analyzed, where the sampling point is to take one trader per market.

#### Sample preparation

Sausages from 5 packs, one pack is taken, then mashed. Then the sausage that had been mashed was weighed as much as 10.0 g, put into a 250.0 ml Erlenmeyer, and labeled. The sample was then immersed in 20.0 mL of 2% ammonia solution (which was dissolved in 70% ethanol) and left overnight. Then the solution was filtered by filtrate using Whatmann filter paper No. 1, and the colored solution was put into an Erlenmeyer. The solution is left in a fume hood for a while, then evaporated on a hotplate. The residue from evaporation is dissolved in 10.0 mL of distilled water containing acid (an acidic solution is made by mixing 10.0 mL of distilled water and 5.0 ml acetic acid 10%). Fat-free wool thread with a length of 20 cm is put into the acid solution and boiled for 10 minutes. The dye will color the wool thread. Then the wool thread is removed and washed with distilled water. Then the wool thread is put into an alkaline solution (10.0 ml of 10% ammonia in 70% ethanol). The woolen thread will release the dye into the alkaline solution (Dawile & Wehantouw, 2013).

#### Qualitative Analysis

##### a. Thin layer chromatography

A 5  $\mu$ l sample was spotted on the TLC plate, then eluted in a vessel containing n-Butanol: Ethyl Acetate: Ammonia (10:4:5) v/v. After the elution, the plate is dried, and the chromatogram is calculated for its  $R_f$  value (Samosir et al., 2018).

## b. Wavelength Comparison

The extracted sample solution is put into a cuvette, and the blank solution is put into the cuvette. Each cuvette is put into a Uv-Vis spectrophotometer, and then scanning is carried out at 400-600 nm. Observe the wavelength of each and compare it to the standard wavelength.

## c. Preparation of Standard Solution

Rhodamin B is weighed as much as 0.1 grams and dissolved with distilled water up to the mark in a 100.0 ml volumetric flask to make a 1000 ppm solution. From a 1000 ppm solution diluted to 100 ppm by pipetting 10.0 ml into a 100 ml volumetric flask, an alkaline solution (10% ammonia dissolves in 70% ethanol) was added to the mark. And diluted again to 20 ppm by pipetting 10 ml into a 50.0 ml volumetric flask of alkaline solution (10% ammonia dissolves in 70% ethanol) to the mark. Then from a 20 ppm solution, concentrations of 0.8 ppm, 1.0 ppm, 1.2 ppm, 1.4 ppm, 1.6 ppm, 1.8 ppm, and 2.0 ppm were made by pipetting 0.4 ml each, 0.5 ml, 0.6 ml, 0.7 ml, 0.8 ml, 0.9 ml, and 1.0 ml into a 10.0 ml volumetric flask then added a basic solution (10% ammonia dissolved in 70% ethanol %) to the boundary mark (Hevira et al., 2020).

## d. Determination of Optimum Wavelength

Pipette 1.0 ml of Rhodamine B solution (concentration 1.4 ppm), add a basic solution (10% ammonia dissolves in 70% ethanol) up to the mark in the 10 ml volumetric flask and homogenize. The maximum absorption was measured at a wavelength of 400-600 nm using a blank. The blank is an alkaline solution (10% ammonia dissolves in 70% ethanol). Then a wavelength versus absorbance curve is made (Arisanti et al., 2019).

## e. Standard curve reading

From 20 ppm of Rhodamin B mains solution, concentrations of 0.8 ppm, 1.0 ppm, 1.2 ppm, 1.4 ppm, 1.6 ppm, 1.8 ppm, and 2.0 ppm were made by pipetting each 0.4 ml, 0.5 ml, 0.6 ml, 0.7 ml, 0.8 ml, 0.9 ml, and 1.0 ml into a 10 ml volumetric flask then added a basic solution (10% ammonia dissolved in 70% ethanol %) to the boundary mark. The absorbance of the standard solution was measured at the wavelength obtained at the maximum wavelength measurement (Devitria & Sepryani, 2016).

## f. Determination of Sample Content

The sample solution obtained from the results of purification was measured for absorption with a Visible Spectrophotometer with a wavelength of 400-800 nm using a basic solution blank (10% Ammonia in 70% ethanol), replicated five times. Rhodamine B levels were determined using the standard curve using the regression equation  $y = a + bx$  (Badan Pengawasan Obat dan Makanan, 2006).

## Data analysis

The research data were analyzed using IBM SPSS 26. The analysis used to test the hypothesis depends on the data distribution, which can be done using the Kolmogorov-Smirnov analysis. If the data is normally distributed ( $p < 0.05$ ), ANOVA analysis can be performed (Sidabutar et al., 2019).

## RESULT AND DISCUSSION

Sausage is made from minced meat and then mashed, seasoned, and put in a symmetrical elliptical sleeve, either made from animal intestines or artificial wrapping (casing). As the food industry develops, more and more meat products are produced, sold, and consumed in a more durable form, such as sausages. To attract consumer interest, synthetic food coloring such as Rhodamin B is added. With the addition of synthetic coloring, the color of the sausage will become brighter and the color more even. Because the color of the sausage is getting more attractive, consumers are more interested in buying the sausage. From the sausage producers' perspective, synthetic dyes can reduce production costs. However, Rhodamine B is a dye whose use is prohibited.

The qualitative test results on seven samples of beef sausage by Thin Layer Chromatography (TLC) and comparing the wavelength of the sample with the standard wavelength of rhodamine B. The qualitative test of Rhodamine B by Thin Layer Chromatography (TLC) was carried out using the mobile phase or eluent (n-butanol: ethyl acetate: ammonia) (10: 4: 5), and the stationary phase used is silica gel 254. In the stationary phase, there is a thin aluminum plate that functions as a place for the absorbent to travel so that the analyte migration process by the solvent can run. After the eluent is made, the solution is saturated first. The saturation process aims to ensure that the mobile phase particles are evenly distributed throughout the chamber so that the spot movement process over the stationary phase by the mobile phase takes place optimally. During the saturation process, the stationary phase is prepared. The aluminum plate used is 10 x 10 cm, with an upper limit of 1 cm and a lower limit of 1 cm. Its function is as a marker of eluent mileage. The plate's lower limit is made so the eluent does not submerge. After that, 5µl of Rhodamine B standard solution was applied, and 5µl of beef sausage sample, which had been extracted-spotted with a capillary tube. The goal is to avoid stain dilation; if too many samples are used, stain dilation will occur. The spreading of the spot will disturb the Rf value. The highlighting is done on the underline that has been made. Then leave it for a while to dry. Next, the plate is inserted with the help of tweezers into a closed chamber containing the eluent with the mobile phase positioned below the line. In raising the mobile phase, the different components of the mixture travel at different rates according to polarity. After approximately 8 cm (or the eluent has reached the upper limit), the plate is removed and dried outside.

The results obtained have fulfilled the requirements because the standard Rf value of Rhodamine B with a sample <0.2. The results can be seen in Table I.

Table I. Comparison of hRf

Replication	Sample	Rf value	Description
1.	A1	0,562	+
	B1	0,650	-
	C1	0,562	+
	D1	0,625	-
	E1	0,625	-
	F1	0,625	-
	G1	0,562	+
2.	A2	0,462	+
	B2	0,387	-
	C2	0,462	+
	D2	0,400	-
	E2	0,387	-
	F2	0,387	-
	G2	0,462	+

The following qualitative test compares the standard wavelength of Rhodamin B with the sample. The goal is to find out the wavelength of the sample and then compare it to the standard wavelength (Figure 1). Three samples were positive for Rhodamine B. The qualitative test results for the wavelength comparison were the same as the qualitative test using Thin Layer Chromatography (TLC).

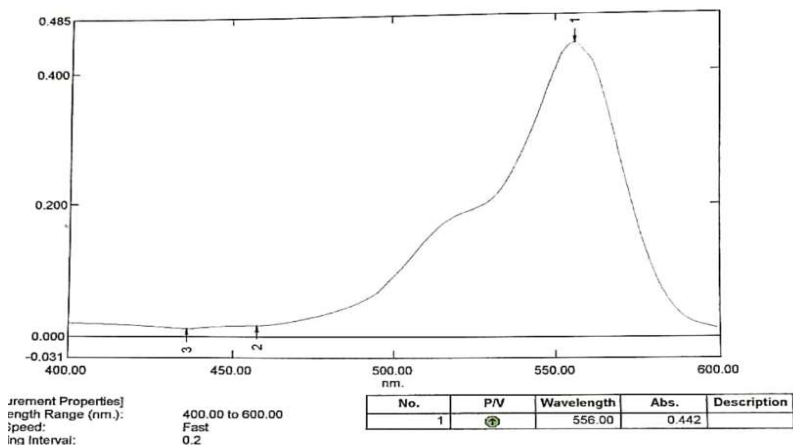


Figure 1. Max wavelength

After conducting qualitative tests, standard curves were made with concentrations of 0.8 ppm, 1.0 ppm, 1.2 ppm, 1.4 ppm, 1.6 ppm, 1.8 ppm, and 2.0 ppm. The purpose of making a standard curve is to measure the absorbance of the standard so that a linear regression equation can be obtained (Figure 2).

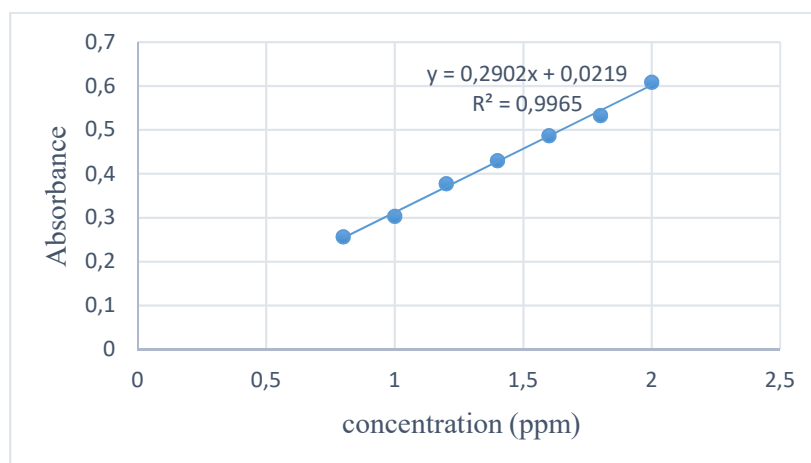


Figure 2. standard curve chart

In the quantitative test of Rhodamine B, namely the determination of Rhodamine levels using the Visible Spectrophotometry method. The choice of this method was based on the fact that Rhodamine B is a colored compound or molecule because it has a chromophore group. The quantity of Rhodamine B color is very sharp due to the presence of 2 auxochrome groups, dimethyl amine. Then because this method is also easy, cheap, and simple, and based on the results of the reading, the maximum wavelength of the Rhodamine B standard is 556.0 nm, so the reading will be more precise if you use Visible Spectrophotometry.

Next is the quantitative test of Rhodamine B, namely by determining the levels of Rhodamine B using the Visible Spectrophotometry method. The Rhodamine B levels in the sample can be determined based on the linear regression equation,  $Y = 0.2902 x + 0.0219$ . Data from the calculation of the content can be seen in table II.

Based on Table II, the average concentration of sample A was 0.67 – 1.06 ppm; in sample C, the average concentration was 0.81 – 0.87 ppm; and in sample G, the average concentration was obtained, namely 0.82 – 1.04 ppm.

Then the data were analyzed with IBM SPSS 26 using the Kolmogorov-Smirnov test. The Kolmogorov-Smirnov test was carried out to find out whether the data were normally distributed or not; in the results of this study, the significance was  $0.122 > 0.05$  (Appendix 16). This result can be said that the data is normally distributed. Then proceed with the homogeneity test; in this study, the results obtained a significance of  $0.000 < 0.05$  (Appendix 17). This shows that the data is not homogeneous.

The data results are normal and homogeneous; an ANOVA test is performed with a 95% confidence level ( $\alpha = 0.05$ ). Obtained a significance value of  $0.411 > 0.05$  (Appendix 18). It can be concluded that there is no difference in the levels of rhodamine B from each sample.

The data from my research showed that the levels of rhodamine B in beef sausage samples were  $1.067 \pm 0.672$ ,  $0.870 \pm 0.818$ , and  $1.046 \pm 0.821$  ppm are much different from the Rhodamine B analysis on steamed sponge samples carried out at the Manado city market using the Visible Spectrophotometry method with levels at seller I 0.0019798 ppm, seller II 0.00119663 ppm, seller III 0.00123415 ppm and seller IV 0.00122575 ppm (Yamlean, 2011).

In addition, research on the analysis of rhodamine b dyes in snacks marketed in the school environment obtained results from 20 samples taken on the market; 6 samples were obtained containing Rhodamine B of 0.3314 ppm, 0.6057 ppm, 0.4675 ppm, 0.4170 ppm, 0.4308 ppm and 0.6521 ppm (Tjiptaningdyah & Bambang Sigit Suchahyo, 2016)

In the study (Dawile & Wehantouw, 2013) on the analysis of crackers in the city of Manado with Visible Spectrophotometry, the results of the 11 samples analyzed obtained one positive sample containing Rhodamin B of 0.28 ppm.

## CONCLUSION

Conclusion: In this study, there were three samples of beef sausage containing Rhodamin B sold at the Yogyakarta City Market with the average levels of Rhodamin in samples A, C, and G respectively  $1.06 \pm 0.67$ ;  $0.87 \pm 0.81$  and  $1.04 \pm 0.82$  ppm.

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