

Analysis of Rhodamine B content in shrimp paste at Ciroyom Market, Bandung City, West Java, Indonesia

Robby Gus Mahardika^{1*}, Farhan Baehaki², Heru Agung Saputra³, Septiani Yusuf⁴

¹Department of Chemistry, Faculty of Engineering, University of Bangka Belitung, Indonesia

^{2,4}Medical Laboratory Technology, Institut Kesehatan Rajawali, Bandung, Indonesia

³Institute of BioPhysio Sensor Technology (IBST) and Department of Chemistry, Pusan National University, Busan, Republic of Korea

ABSTRACT

According to a regulation number 722/MENKES/Per/IX/88 of the Minister of Health of the Republic of Indonesia, Rhodamine B is one of dangerous dyes, and its use in food products is prohibited. Rhodamine B can cause adverse health effects including irritation, impaired liver function, and liver cancer. Although it has been banned, the use of Rhodamine B is still prevalent in the community's food supply, including shrimp paste. This can be observed in some of the shrimp pastes sold at the Ciroyom market in Bandung, which are bright red, uneven, and comprised of lumps. This study aims to determine the Rhodamine B content of shrimp paste sold at Ciroyom Market in Bandung, West Java, Indonesia. This research is descriptive in nature and employed a total sampling technique. Rhodamine B was analyzed in ten samples of commercially available red shrimp paste using a UV-Vis Spectrophotometer with a 565 nm wavelength. The results indicate that Rhodamine B was found in all samples, with concentrations ranging from 1.79 ppm to 3.999 ppm. Therefore, it can be concluded that widespread abuse of Rhodamine B in food ingredients persists among the residents of Bandung City, West Java, Indonesia.

ARTICLE INFO

Received: 25 April 2022

Accepted: 31 May 2022

Published: 6 June 2022

KEYWORDS

Rhodamine B, Shrimp paste, UV-Vis Spectrophotometer

INTRODUCTION

There is a source of energy for the human body in food because it contains compounds that are essential for human growth and development. Food is a fundamental need for all humans (Purwati et al., 2018; Saeffurqon et al., 2017). Effendi (2012) states that organic ingredients dominate food composition. Currently, food manufacturers are concerned not only with the nutrient content of their products, but also with packaging

that is convenient, attractive, and long-lasting. Generally, additives are added to these foods to preserve and flavor the product. (Saeffurqon et al., 2017).

According to Regulation of the Minister of Health of the Republic of Indonesia No. 1168/MenKes/Per/X/1999 concerning Amendments to the Regulation of the Minister of Health of the Republic of Indonesia No. 722/MenKes/Per/IX/1988 concerning Food Additives, food additives are non-food substances added to food to improve its quality. These substances may or may not have nutritional value. Food additives are used for a variety of purposes, one of which is to extend the shelf life of food by inhibiting the growth of microorganisms that degrade food or preventing chemical reactions that degrade food quality, as well as to enhance the flavor of food and produce more appetizing colors, aromas and flavor (Alsuhendra & Ridawati, 2013; Sucipto, 2015).

A dye is one of the most commonly used food additives. Dye additives are used to improve or add color to food to make it more visually appealing (Sucipto, 2015). There are two types of dyes in use: natural and synthetic (Utama et al., 2021). Natural dyes include caramel, chocolate, pandan leaves, and turmeric, which are derived from animals and plants. While synthetic dyes are obtained through chemical processes that are artificially induced. Diamond blue, HT chocolate, erythrosine, FCF green, S green, indigotin, carmoisine, FCF yellow, quinoline yellow, groove red, Ponceau 4R, and tartrazine are common artificial dyes (Sembel, 2015). A number of dyes, including methanol yellow and Rhodamine B, are prohibited for use in food. Both of these dyes are textile dyes, so their use in food poses a health risk (Alsuhendra & Ridawati, 2013; Saeffurqon et al., 2017).

Rhodamine B is frequently misapplied in the production of food, including shrimp paste. Shrimp paste is an ingredient derived from fermented shrimp or fish that is solid, smells, and is either red or brown in color. However, it is suspected that some red shrimp paste products contain Rhodamine B. Several researchers have investigated the analysis of the Rhodamine B concentration in shrimp paste. (Amir & Mahdi, 2017) state that in Makassar City, Indonesia, they found samples of shrimp paste containing Rhodamine B on the market. Ingestion of sufficient quantities and frequency of foods containing Rhodamine B will cause skin irritation, eye irritation, digestive irritation, respiratory irritation, poisoning, liver function disorders, and liver cancer (Alsuhendra & Ridawati, 2013; Hasanah et al., 2014; Saputri et al., 2018; Sonker et al., 2020; Sucipto, 2015). Rhodamine B-containing foods are characterized by their bright red color, sometimes uneven hue, and the presence of color lumps (Kamaruzzaman et al., 2021; Purniati & Rama, 2015; Sucipto, 2015).

The Ciroyom market is one of the markets in Bandung that sells a variety of shrimp paste. A portion of the shrimp paste sold on the market is bright red, uneven, and contains lumps. The shrimp paste manufacturer added Rhodamine B to achieve the desired hue, making the shrimp paste more appealing. Another factor is the producers' ignorance of the dangers of Rhodamine B dye and its lower cost (Amir & Mahdi, 2017; Cahyadi, 2012). On the basis of these conditions, it is necessary to conduct research on the concentration of Rhodamine B in red shrimp paste sold at the Ciroyom market in Bandung, West Java, Indonesia.

LITERATURE REVIEW

Shrimp paste

The process of fermentation is utilized to produce shrimp paste, one of the fishery products. According to Murniyati & Sunarman (2014), Fermentation is a process in which meat is broken down by enzymes that produce beneficial results. Fermentation and putrefaction are similar processes, but fermentation produces substances with a specific flavor and aroma that people enjoy. This distinctive flavor and aroma can be detected in peda fish, shrimp paste, fish sauce, etc. Class I shrimp paste is made with rebon shrimp, class II shrimp paste is made with sea rebon, class III shrimp paste is made with a mixture of rebon shrimp and sea fish, and class IV shrimp paste is made with shrimp heads and fish (Suwandi et al., 2017). The characteristics of shrimp paste containing Rhodamine B are its red color, the presence of lumps, and its uneven texture.

Rhodamine B

Rhodamine B is a colorant that comes in the form of odorless, green or reddish-purple crystals that are easily soluble in water and produce a bluish-red hue and strong fluorescence (Aprilianintyas, 2020; Wang et al., 2015). Not only is it easily soluble in water and ether, but it is also difficult to dissolve in alcohol, HCl, and NaOH. Its empirical formula is $C_{28}H_{31}ClN_3O_3$. Rhodamine B's chemical name is [9-(2-carboxyphenyl)-6-diethylamino-3-xanthenylidene] dimethylammonium chloride. It has a molecular weight of 479.02, is soluble in water at 50 g/L, and in aqueous acetic acid at 400 g/L. It has a melting point between 210 and 2110 degrees Celsius, which will result in decomposition and the destruction of Rhodamine B material (Amir & Mahdi, 2017; Praja, 2015; Prayoko & Thristy, 2017).

Both diethylamino-phe zero and phatalic anchidria are extremely toxic to humans and are used to create rhodamine B. Rhodamine B is frequently used to color paper, wool, and silk. Ingestion of large quantities of Rhodamine B in a short period of time can cause acute poisoning symptoms. Rhodamine B has a lethal dose of 500 mg/kg body weight. Rhodamine B added to food can irritate the digestive tract and cause symptoms of poisoning, including red or pink urine. Inhaled Rhodamine B can cause respiratory tract irritation and other health issues. Rhodamine B can cause irritation such as eye irritation, which is characterized by redness of the eyes and accumulation of fluid or edema in the eyes, when exposed to the skin (Prayoko & Thristy, 2017).

Toxicity of Rhodamine B

According to the Drug and Food Control Agency of the Republic of Indonesia (2008), There are both short-term and long-term effects of Rhodamine B. a) if inhaled dust or mist it causes irritation to the respiratory tract with symptoms of cough, sore throat, difficulty breathing, and chest pain; b) if the dust, powder, or solution in contact with the skin can cause irritation to the skin, such as redness and pain; and c) if contact with the eyes can cause severe injury, including conjunctival edema, hyperemia, and pus discharge, resulting in complete blurring and even tissue damming. Possible long-term effects include skin inflammation and

allergic reactions. Long-term use of Rhodamine B in food will lead to impaired liver function and cancer.

Process of absorption, distribution, metabolism, and excretion of Rhodamine B

Rhodamine B is extensively absorbed by the gastrointestinal tract and metabolized in dogs, cats, and rats, with only 3 to 5 percent of the administered dose of Rhodamine B remaining unchanged in urine and feces (Drug and Food Control Agency of the Republic of Indonesia, 2008). Due to the polar nature of Rhodamine B, its metabolism has become one of the causes of systemic organ damage. Rhodamine B, which is not metabolized by the liver due to its polar nature, will spread through the bloodstream by interacting with amino acids in blood globulin, with the liver being the organ at greatest risk and target (Drug and Food Control Agency of the Republic of Indonesia, 2008; Huang et al., 2020).

When Rhodamine B enters the body, the liver metabolizes it. When Rhodamine B enters the body orally, it will undergo various processes in the gastrointestinal tract, just as food does in general (Su et al., 2015; Webb & Hansen, 1961). When ingested, Rhodamine B (tetraethyl-3', 6'-diaminofluorane) is de-ethylated into multiple metabolites, including 3', 6'-diaminofluoran and N, N'-diethyl-3', 6'-diaminofluoran. Another Rhodamine B metabolite is the monomethylated form. Rhodamine B cannot be metabolized by the body and undergoes no other metabolic process besides de-ethylation (Webb & Hansen, 1961). Rhodamine B-glucoronide, an acyl-type metabolite, is another possible Rhodamine B metabolite (Braakman et al., 1989).

Small amounts of Rhodamine B can be expelled from the body. Only a few percent of people. The unchanged form of Rhodamine B can be found in urine and faeces (Webb and Hansen, 1961). One of two fluorescent metabolite forms can be produced from Rhodamine B samples incubated with cecal microflora in about 30% of the cases (European Food Safety Authority, 2005).

METHODS

A descriptive approach was used in this study, which is a strategy for describing or analyzing a research result but not drawing broad conclusions (Sugiyono, 2009). This study aimed to find out how much Rhodamine B was in shrimp paste sold in Bandung, Indonesia's Ciroyom Market. The study was carried out at the Rajawali Health Institute's Laboratory of Applied Chemistry and Toxicology in Bandung, Indonesia, using a UV-Vis spectrophotometer.

Sample preparation

Referring to the procedure followed, the sample preparation was completed as suggested by Amir & Mahdi (2017). To acidify the shrimp paste, 5 mL of a 10% acetic acid solution was added to a 100 mL beaker containing 1 gram of the sample. In addition, the beaker is filled with the wool thread. Beaker is brought to a boil, then cooled until it is at a comfortable temperature. Using distilled water, wool threads are rinsed and then placed in a new beaker. It was heated until the color on the wool thread faded, then 25 mL of a 10% ammonia solution was added to the beaker and heated.

Preparation of 100 ppm Rhodamine B solution

There were 0.01 grams of Rhodamine B weighed out and mixed with 25 milliliters of HCl solution to make 25 milliliters of solution. On top of that, 0.01 N hydrochloric acid solution was poured into a 100-mL volumetric flask.

Creating standard curves

In order to create a standard curve, standard solutions with concentrations of one, two, three and four parts per million were created. Diluting Rhodamine B 100 ppm solution with the following calculations produces the solution.

- Standard solution 1 ppm

$$100 \text{ ppm} \times V_1 = 1 \text{ ppm} \times 100 \text{ ml}$$

$$V_1 = \frac{1 \text{ ppm} \times 100 \text{ ml}}{100 \text{ ppm}}$$

$$V_1 = 1 \text{ ml}$$

- Standard solution 2 ppm

$$100 \text{ ppm} \times V_1 = 2 \text{ ppm} \times 100 \text{ ml}$$

$$V_1 = \frac{2 \text{ ppm} \times 100 \text{ ml}}{100 \text{ ppm}}$$

$$V_1 = 2 \text{ ml}$$

- Standard solution 3 ppm

$$100 \text{ ppm} \times V_1 = 3 \text{ ppm} \times 100 \text{ ml}$$

$$V_1 = \frac{3 \text{ ppm} \times 100 \text{ ml}}{100 \text{ ppm}}$$

$$V_1 = 3 \text{ ml}$$

- Standard solution 4 ppm

$$100 \text{ ppm} \times V_1 = 4 \text{ ppm} \times 100 \text{ ml}$$

$$V_1 = \frac{4 \text{ ppm} \times 100 \text{ ml}}{100 \text{ ppm}}$$

$$V_1 = 4 \text{ ml}$$

- Standard solution 5 ppm

$$100 \text{ ppm} \times V_1 = 5 \text{ ppm} \times 100 \text{ ml}$$

$$V_1 = \frac{5 \text{ ppm} \times 100 \text{ ml}}{100 \text{ ppm}}$$

$$V_1 = 5 \text{ ml}$$

For each standard solution, the UV-Vis Spectrophotometer at 565 nm was used to measure absorbance. As the concentration and absorbance data were plotted onto a graph, $y = ax + b$ and the value of R^2 could be determined.

Measurement of Rhodamine B levels in the sample

To prevent false positive measurements, the wool yarn damping solution was filtered and 100 mL of distilled water was then added to the filtrate. In addition, the Rhodamine B concentration in the sample was measured with a UV-Vis Spectrophotometer at a wavelength of 565 nm.

RESULTS

Standard curve

Based on a standard series of known concentrations, the standard solution curve is constructed. The absorbance of the standard series solution was measured, and the results were expressed as a standard curve. Using a UV-Vis Spectrophotometer, the standard solution was measured with a wavelength of 565 nm in accordance with the solution series. Table 1 displays the measured absorbances of five rows of different concentrations of standard solutions at a wavelength of 565 nm.

Table 1. Absorbance of Rhodamine B standard solution

Concentration (ppm)	Absorbance
1.0	0.005
2.0	0.010
3.0	0.024
4.0	0.035
5.0	0.048

The data from Table 1 are graphed to obtain the equation of the line $y = mx + c$ and the value of R^2 . The obtained standard curve is shown in Figure 1.

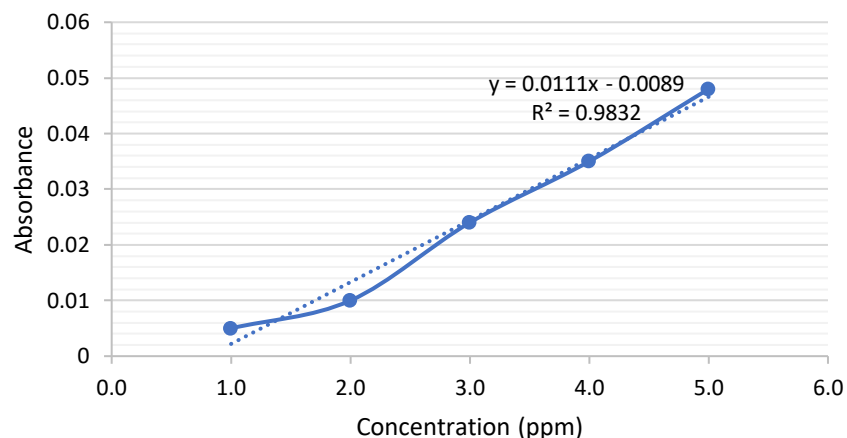


Figure 1. Standard curve of Rhodamine B standard solution

Based on Figure 1, the regression coefficient value (R^2) is 0.9832, indicating that the R^2 value is close to ideal with an error rate below 1 percent. Therefore, the resulting equation, $y = 0.0111x - 0.0089$, can be utilized to calculate the Rhodamine B concentration in the shrimp paste sample.

Rhodamine B Levels in shrimp paste samples

In this study, the determination of Rhodamine B levels was performed twice for each sample, and then the results were averaged. Table 2 outlines the determination of Rhodamine B concentrations. The levels of Rhodamine B in shrimp paste as determined by a UV-Vis Spectrophotometer are displayed in Table 2. Based on measurements of Rhodamine B levels in shrimp paste sold at the Ciroyom market in Bandung, Indonesia, all positive samples of shrimp paste contained Rhodamine B. The highest concentration of Rhodamine B was found in sample E at 3,999 ppm, while the lowest concentration was found in sample J at 1,792 ppm.

Table 2. Results of measuring levels of Rhodamine B in the sample

Sample Code	Absorbance	Concentration (ppm)
A	0.019	2.513
B	0.022	2.838
C	0.017	2.333
D	0.020	2.648
E	0.035	3.999
F	0.031	3.594
G	0.021	2.738
H	0.015	2.198
I	0.018	2.468
J	0.011	1.792

DISCUSSION

Observation of the physical condition of the shrimp paste

The characteristics of foods containing Rhodamine B are that they are bright red, the color is sometimes uneven, and the product contains color lumps (Purniati & Rama, 2015; Sucipto, 2015). Based on the observation of ten samples of shrimp paste, all shrimp paste is observed to be bright red. The shrimp paste samples sold at the Ciroyom market in Bandung were suspected of containing Rhodamine B based on the aforementioned characteristics.

Standard curve

The measurement of the shrimp paste sample began with the measurement of the standard solution, which serves as a measurement standard for the analyte. The results were then plotted on a standard curve to determine the regression value of the curve. From a 100 ppm stock solution of Rhodamine B, five series of standard solutions with variations of 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm were used to measure standard

solutions. At 565 nm, a UV-Vis spectrophotometer was used to conduct measurements.

The function of the curve in this study is to determine the equation of a linear line. Using the linearity test of regression determination (R^2) and the standard curve, the correlation coefficient is calculated. The equation for the line depicted in Figure 1 is obtained by plotting the absorbance value of the standard solution of Rhodamine B against the concentration of the standard series of Rhodamine B. The correlation coefficient indicates a significant relationship between solution concentration (x-axis) and absorbance (y-axis) (y-axis). The ideal linear relationship exists when R^2 equals 1 or -1 (Harmita in Saputra, 2016). The obtained value for the regression coefficient is $R^2 = 0.9832$. This indicates that the R^2 value is close to the ideal value and that the resulting equation can be used to determine the Rhodamine B levels in the shrimp paste sample (Baehaki et al., 2020, 2021).

Rhodamine B levels in the sample

The results of a study on 10 samples of shrimp paste sold at Ciroyom Market in Bandung, Indonesia, with varying concentrations of Rhodamine B are presented in Table 2. Based on the Regulation of the Minister of Health of the Republic of Indonesia Number 722/MENKES/Per/IX/88 concerning food additives, Rhodamine B is prohibited in food because it poses a health risk. According to the ten samples that were analyzed, all of them tested positive for Rhodamine B, even at levels high enough to render the shrimp paste unusable and potentially hazardous. According to the Drug and Food Control Agency of the Republic of Indonesia (2008), Rhodamine B's toxic effects can cause respiratory irritation, eye irritation, and red or pink skin irritation in the short term, and impaired liver function and liver cancer in the long term.

Due to its polar nature, the metabolic process of Rhodamine B can be one of the causes of systemic organ damage. Rhodamine B, which is not metabolized by the liver, will spread through the bloodstream and interact with amino acids in blood globulin due to its polar nature. The liver is the primary organ at risk and target (Drug and Food Control Agency of the Republic of Indonesia, 2008; Sunarti & Bhakti, 2014). Rhodamine B will be primarily metabolized by the liver upon entry into the body. When Rhodamine B enters the body orally, it undergoes the same processes in the gastrointestinal tract as food in general (Webb & Hansen, 1961). Very little Rhodamine B is excreted unchanged in urine or feces; only 3 to 5 percent of Rhodamine B can be found in such forms (Webb & Hansen, 1961).

CONCLUSION

Research on red shrimp paste sold at the Ciroyom market in Bandung, West Java, Indonesia, revealed that all positive samples contained Rhodamine B, with the lowest level in sample J and the highest level in sample E, respectively. On the basis of these data, it can be concluded that producers continue to abuse Rhodamine B in food the most. This research data should serve as the foundation for Indonesian food supervisory agencies to further tighten rules and oversight of food circulating in the community. Thus, the safety of food for human health is increased.

ACKNOWLEDGEMENTS

The authors would like to thank Institut Kesehatan Rajawali for assisting and facilitating the execution of this study.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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