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UV-VIS Spectrophotometer Analysis of Rhodamine B in Shrimp Paste

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ABSTRACT

Fermented shrimp are used to create a condiment known as shrimp paste. To cut costs, shrimp paste manufacturers frequently use rhodamin b as a food additive in their products, even though it is illegal to do so. The use of synthetic rhodamine b in food is prohibited by Regulation of the Minister of Health of the Republic of Indonesia Number 239/Menkes/Per/V/85 regarding certain dyes declared as hazardous materials. When used frequently, rhodamine b can lead to hepatic toxicity, renal swelling, and cancer. This investigation measured how much rhodamine b can be found in shrimp paste. This research employed a non-probability, purposive sampling approach. The samples were also analysed quantitatively with a UV-Vis Spectrophotometer and qualitatively with reagents. Each sample of shrimp paste tested positive for rhodamine b. At the same time, the concentrations of rhodamine b in samples A (0.274 mg/g), B (0.204 mg/g), and C (0.174 mg/g) were determined using a UV-Vis Spectrophotometer set to max 557 nm. There was rhodamine b in all three of the shrimp paste samples tested.

Keywords: Rhodamin b, Shrimp paste, Spektrofotometer UV-Vis

INTRODUCTION

Rhodamin B is a synthetic dye prohibited from being used in food under the Regulation of the Minister of Health of the Republic of Indonesia regarding certain dyes designated as hazardous materials. Certain dyes are substances used to colour or improve the colour of materials or goods[1]. Rhodamine B is a prohibited dye often found in food, for example, shrimp paste, crackers, syrup, and sausages[2]. Rhodamine B is a green crystal or reddish-purple powder, very soluble in water, produces a bluish-red solution and fluoresces strongly when diluted. It is soluble in ethanol and sparingly in dilute acids and alkaline solutions. Additionally, it is soluble in strong acids, forming a compound with a pink antimony complex soluble in isopropyl ether[3].

Various industries employ the dye rhodamin B, including paint, textiles, and paper. This dye is carcinogenic and might irritate the respiratory tract. Liver injury is possible at high doses of rhodamine B[4]. Long-term exposure to rhodamine B in food has been linked to liver damage, tumour development, and cancer. Acute symptoms of rhodamine B poisoning occur, however, when significant doses of the substance are consumed rapidly[5]. One type of fermented fish product is shrimp paste, and it's typically produced with little shrimp because of its rough texture, dense shape, and characteristic aroma[6].

In 2011, the Food and Drug Administration of Indonesia sampled and tested snack food for schoolchildren from 866 elementary schools spread across 30 cities in Indonesia. There were 4,808 snack food samples taken, 1,705 (35.46%) of which did not meet food quality safety requirements. The test parameters for prohibited food additives, namely borax and formalin, were tested on 3,206 snack food products for school children. It was discovered that 94 (2.93%) samples contained borax, and 43 (1.34%) samples contained formalin. The results of testing the non-food dye test parameters (rhodamine B) on 3,925 school-aged snack food products revealed that 40 (1.02%) samples contained rhodamine B[7]. According to the findings of a previous study, Amir and coworkes[8], which used a rhodamine B tester kit, up to 60% of shrimp paste marketed in Makassar City contained rhodamine B at levels ranging from 11.81 to 19.05 ppm. Meanwhile, according to the findings of a study conducted by Mamay and colleagues[9] on qualitative testing using the TLC method and quantitative testing using a UV-Vis Spectrophotometer, there were four samples of shrimp paste tested that were positive red containing rhodamine B with concentrations ranging from 57, 55-222.5 mg/kg.

Based on previous research data indicating that the use of prohibited food additives such as rhodamine B is still occurring. We are interested in conducting additional research on the analysis of rhodamine B in non-registered shrimp paste products using the UV-Vis Spectrophotometer method.

METHODS

Chemicals and sample preparations

All chemicals and reagents used were of analytical grade and were used as received without any further purification from Sigma-Aldrich. Non-registered shrimp paste products were purchased and collected from stores in Indonesia, and randomly three samples were chosen for further analysis.

Qualitative analysis of rhodamine B

Two grams of shrimp paste was weighed and then crushed with a mortar and pestle until smooth. The shrimp paste was placed in a 10 mL volumetric flask, followed by 5 mL of 10% NaOH solution, 2 mL of petroleum ether solution, and shaking to extract the sample, as evidenced by the formation of two separate layers, the upper layer being a clear ether solution and the lower layer being brown water. After allowing the solution to stand for a few minutes to separate the two layers, the ether layer was pipetted into a test tube, 2 ml of 10% HCl solution was added, and the test tube was slowly shaken. Examine whether the colour of the ether solution changes from clear to red after adding 10% HCl solution. The positive result showed a red colour on the test[10].

Qualitative analysis of rhodamine B by using UV-Vis Spectrophotometry

Standard rhodamine B 100 g/mL solution was pipetted into a 10 mL volumetric flask and diluted with 0.1 N HCl solution to prepare a variety of concentrations of 2, 3, 4, 5, and 6 g/mL. The maximum absorption was measured at a 400-800 nm wavelength, and the linear graph of the concentrations against absorbance was plotted. One gram of shrimp paste was prepared and placed in an evaporating cup with seven drops of 0.1 N HCl and 15 mL of methanol. The sample was heated in a water bath for 15 minutes and centrifuged for 4 minutes. In addition, anhydrous Na-sulfate was added and filtered into a 50 mL volumetric flask with 0.1 N HCl. The final sampleS (A B C) were measured with a UV-Vis Spectrophotometer at a maximum wavelength.

Determination of rhodamine B levels is as follows:

$$K = \frac{X\ (mg/mL).V\ (mL).Fp}{BS\ (g)}$$

K: Total level of rhodamine B in the sample (mg/g)

X: Sample concentration (ppm)

V: Sample volume (mL)

Fp: Dilution factor (mL)

BS: Sample weight (g)

RESULT AND DISCUSSION

Rhodamine B is a component of synthetic dyes that have been banned from use in food, even in trace levels, since it is carcinogenic and can stimulate the formation of cancer cells. This study revealed the misuse of rhodamine b dyes in some shrimp paste products sold in Indonesia. Three market samples (designated A, B, and C) were used in this study. In cases where unregistered shrimp paste was used to pick the sample, the shrimp paste had a brighter sheen, lacked identifying information like a code, label, or brand, and had a more noticeable identity.

The existence of rhodamine B content was determined by observing a colour change in each sample submitted to the qualitative test. When a 10% HCl solution was applied to samples containing rhodamine B, the colour changed from clear to red. Sample colour change suggested a response between 10% HCl and rhodamine B. When NaOH was included, it acted as an alkaline solvent. Petroleum ether can remove grease and extract rhodamine b colour in a primary setting. An acid test for rhodamine B's presence can be performed with HCl, a powerful acidic chemical substance. Qualitative reagent testing revealed a reddening of samples A, B, and C, indicating the presence of rhodamine B (Fig. 1).

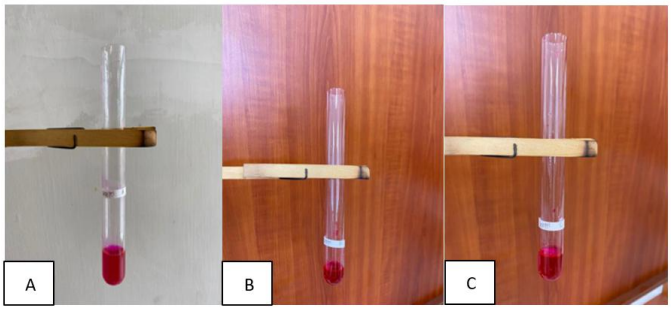

Fig 1. Qualitative analysis of shrimp pastes A B, and C

To further quantify the rhodamine B concentration in the shrimp paste samples, a UV-Vis spectrophotometer was used to analyse the samples' absorbance spectra. The investigation was done by measuring the maximum wavelength from 400-800 nm using a standard solution of rhodamine B at a concentration of 3 g/ml. Rhodamine B is a coloured compound, precisely a bluish-red colour. Therefore, it was read in the visible range of 400-800 nm to determine the maximum wavelength. The maximum wavelength obtained was 557 nm (Fig 2).

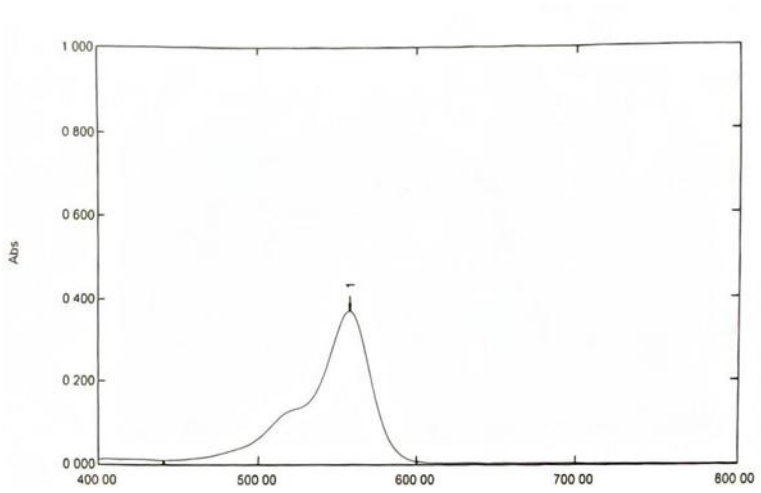

Fig 2. The maximum wavelength of rhodamine B

Following that, a calibration curve was created for a standard solution of rhodamine B 100 g/ml with concentrations of 2, 3, 4, 5, and 6 g/ml and measured at 557 nm. A linear equation y = 0.06329x + 0.17238 with a correlation coefficient r = 0.99943 was obtained (Fig 3.). The detection limit (LOD) and quantitation limit (LOQ) can be determined by getting the regression equation, where each value is obtained for the detection limit of 3.88 g/mL and the quantitation limit accepted is 12.92 g/mL.

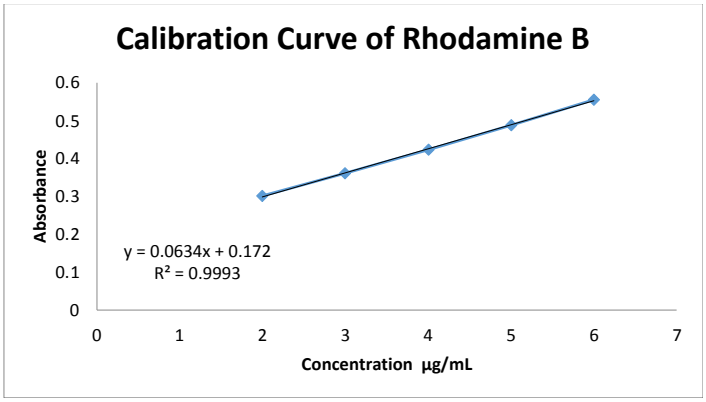

Fig 3. Calibration curve of rhodamine B

Furthermore, the levels of rhodamine B in the shrimp paste samples labelled A, B, and C was determined. When determining the concentration of the shrimp paste samples, 0.1 N hydrochloric acid (HCl) was added to destroy the compounds in the samples and stabilise rhodamine B. Therefore, it did not change from the ionised to neutral form. Moreover, 15 mL of methanol was added as a solvent because rhodamine B is highly soluble in alcohol. The sample was heated and centrifuged for 4 minutes. Anhydrous Na-sulfate was added to clarify the solution so that the light from the UV-Vis Spectrophotometer could penetrate it and be detected. The absorbance of the sample solution was then measured using UV-Vis spectrophotometry at a maximum wavelength of 557 nm. Table 1 shows the sample levels obtained from the experimental results of shrimp paste samples at a concentration of 100 µg/mL.

Table 1. rhodamine B level of A B C

Samples	**Rhodamine B Level (mg/g)**
A	0.274
B	0.204
C	0.174

Rhodamine B is a chemical compound that binds with chlorine (Cl), a reactive and dangerous inorganic compound. The chlorine atom (Cl) is a member of the halogen group. Because of the elements nitronium (N+) and chlorine (Cl) contained, the nature of these halogens will cause toxic and carcinogenic effects or promote the growth of cancer cells if used continuously. In the reaction for the formation of rhodamine B or the synthesis of dyes, chlorine ions are bound. Fluorescein is produced by phthalic anhydride and resorcinol in the presence of zinc chloride. This reaction will make rhodamine B if resorcinol is replaced with N-N-diethylaminophenol.

The substances in rhodamine B will accumulate in the body. Like many other harmful chemicals, the liver will work hard to break them down so they can be excreted from the liver to the circulatory system, which then to the kidneys; the kidneys must work hard to expel these substances from the body. The liver's ability to break down these substances is limited, resulting in liver damage, leading to kidney damage when the liver can no longer process toxic substances produced by dangerous chemicals like rhodamine B[11].

CONCLUSIONS

The government should be concerned about the rising demand for shrimp paste as an ingredient in food products because of the necessity to regulate the safety and quality of products on the market. This research showed that manufacturers still use rhodamine B in shrimp paste without considering the consequences for customers. Regulation of the Minister of Health of the Republic of Indonesia Number 239/Menkes/Per/V/85 addressing specific dyes declared as hazardous materials must be fully implemented by the Indonesian FDA.

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