

## **Qualitative Analysis of Rhodamine B Dye on Usek Crackers Sold by Producers in Kasepuhan Village, Batang District**

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### **Abstract**

This study aimed to evaluate the presence of Rhodamine B dye in usek crackers produced by local manufacturers in Kasepuhan Village, Batang District, Batang Regency. The use of Rhodamine B in food products poses a serious health risk to the community, as it is a banned dye due to its negative impact on human health. The study used the Wool and Thin Layer Chromatography (TLC) method or called KLT method to identify the presence of Rhodamine B in the samples. The results showed that 4 samples tested were positive for Rhodamine B, indicating that food manufacturers still use Rhodamine B dye in their products. This discovery highlights the importance of regular monitoring and evaluation to ensure the safety and quality of food products. The study's findings can be used to inform the public about the risks of consuming Rhodamine B-containing food products and to encourage the use of natural dyes in food production.

**Keywords:** Usek Crackers, Rhodamin B, Kasepuhan Batang Village

### **Introduction**

Currently, in Indonesia, there are many consumer conflicts, especially in the food sector. Among them is the problem of misuse of Food Additives carried out by food producers who are not suitable for using the original function of Food Additives. The reason manufacturers use food additives that are not suitable for food is to make it look more attractive, cheaper, and affordable. One of the discrepancies in the maker's use of food additives is the use of textile dyes in food (Juliana et al., 2023).

In general, dyes are divided into 2 types, namely natural dyes and synthetic dyes. Natural dyes are dyes that come from natural materials such as plants, minerals, and animals. Meanwhile, synthetic dyes are dyes derived from chemicals used for textile dyes, paints, and others. Synthetic dyes have a bad impact on human health, such as irritation in the skin, irritation in the eyes, carcinogenic, mutagenic to liver damage (Hevira et al., 2020).

Based on the regulation of the Minister of Health of the Republic of Indonesia No.239 / Menkes / Per/V / 85, it was decided that there are around 30 dangerous synthetic dyes that are not allowed to be used in food and beverage products. Rhodamine B includes one origin error of 30 dyes whose use is stopped in food products (Simamora & Puspawati, 2023). Rhodamine B is a dye in the form of crystalline powder, which has no odor and a purplish red color; the solution form is bright red, similar to neon (fluorescent)

(Saviello et al., 2018). Rhodamine B has side effects when used in food products, such as poisoning, skin irritation, eye irritation, digestive tract disorders, and liver disorders, and can cause cancer (Wu et al., 2022).

Although the use of Rhodamine B in food has been banned by the government, there are still many manufacturers who use Rhodamine B dye. According to Tribunnews news (6/7/2022), BPOM Semarang held a hearing in the Batang Regency area. The results of the krupuk use sampling test in Batang Regency, Central Java, namely 4 samples tested, showed the presence of Rhodamine B content or positive for Rhodamine B. This discovery shows that food manufacturers still use Rhodamine B dye in their products. Use crackers are processed crackers that cook using sand without using a frying process with oil (Sulistri, Sunarsih, Utama, & Moseki, 2020). Culinary characteristics that contain Rhodamine B mean a more striking color, sometimes clear gloss, hues that look uneven, or there are lumpy colors (Ridjal & Kasma, 2022).

Thin Layer Chromatography is a simple way to identify the introduction of a compound. The TCL or KLT method is useful in separating a compound on the basis of a 2-phasedistribution difference that includes the stationary phase and the motility phase. Researchers chose this KLT method because the implementation method is simple and does not require a long time (Nindyasari & Asyifiradayati, 2024).

In addition to using the KLT method, another method that can be used to identify the presence of Rhodamine B compounds is the Wool Yarn method. Wool yarn will later release dyes, and the dye or dye will enter the alkaline solution. Later an alkaline solution will be obtained, which is then concentrated, and then it will be used for sample footage in the next analysis (Saviello et al., 2018).

Based on this background, usek crackers as a food product that is quite much liked by the community, especially in the Batang area and its surroundings, must be ensured its quality. People need quality food products and good quality to ensure the health or safety of the food purchased.

The choice of location in Kasepuhan Village, Batang District, Batang Regency as a place of research because they wanted to know whether there was an addition of Rhodamine B dye to usek crackers produced and sold by producers in the area or not. In addition, a hearing has been held by the Health Office regarding the content of Rhodamine B in usek crackers, which is another reason for choosing the location.

Problems arising from the presence of Rhodamine B dye in usek crackers pose a serious health risk to communities in Batang and its surroundings. To prevent consumption of usek crackers containing Rhodamine B and protect public health, a study is needed that aims to qualitatively analyze the content of Rhodamine B dye in usek crackers sold by producers in Kasepuhan Village, Batang District, Batang Regency.

The formulation of the problem from this study is whether usek crackers produced by producers in Kasepuhan Village, Batang District, contain Rhodamine B dye. This study aims to identify the presence of Rhodamine B dye in usek crackers produced by producers in Kasepuhan Village, Batang District, using the Wool and Thin Layer Chromatography (TLC) method. It is hoped that the results of this study can provide

information to the public about the risks of using Rhodamine B dye by dishonest producers in food products, especially usek crackers sold in Kasepuhan Village, Batang District, Batang Regency.

### **Research Methods**

The type of research used is descriptive observational, which aims to provide an overview of the identification of Rhodamine B dye in usek crackers sold by producers in Kasepuhan Village, Batang District. The identification method is carried out qualitatively using wool methods and Thin Layer Chromatography (TLC).

The population in this study was all usek crackers sold by producers in Kasepuhan Village, Batang District. The sampling process is carried out to select a number of samples from the population in order to describe the overall characteristics of the population. The sampling technique used is saturated samples.

The research site consists of two places, namely sampling and sample research: a) The sampling place in this study is in Kasepuhan Village, Batang District. b) The place where sample research is carried out is the Chemistry Laboratory, Faculty of Pharmacy, University of Pekalongan. The time of this study is taken from the preparation of proposals to data collection starting in October to December 2023.

Data analysis is carried out, covering several stages. The initial stage is descriptive data analysis. Namely, data is collected, processed, and analyzed based on existing phenomena or data characteristics. The second stage is to identify the presence of Rhodamine B content qualitatively, which is carried out using the wool and KLT method. The identification of both methods was carried out to find out and conclude whether use crackers contain Rhodamine B or not. In the wool method, a positive result is characterized by the non-leaching of the red color inherent in the woolen thread.

In the identification process using TLC, observations were made on spots that appeared during the chromatography process under 254nm UV light. Rhodamine B will exhibit yellow fluorescence when observed under 254nm UV light and will be visually pink (POM Team, 2014). After looking at the fluorescence of Rhodamine B in usek crackers, its R<sub>f</sub> value was calculated and compared to the standard R<sub>f</sub> value. A sample is considered to contain Rhodamine B if the R<sub>f</sub> value obtained is equal to or close to the standard R<sub>f</sub> value of Rhodamine B.

### **Results and Discussion**

In the current era of scientific and technological progress, there is abuse in the addition of food ingredients that can endanger health, such as Rhodamine B dye, which is often added to food products, including crackers. This study aims to provide an overview of the content of Rhodamine B dye in usek crackers, using identification methods using wool and KLT methods. The sampling process was carried out in Kasepuhan Village, Batang District, using saturated sampling techniques. From this process, four samples of usek crackers from different manufacturers were obtained. Kasepuhan Village, Batang District, was chosen because of its reputation as one of the

leading producers of usek crackers in the Batang region. Furthermore, each sample will be replicated or repeated three times in each identification method.

The sample used is red usek crackers that have been processed and are ready to sell. Before identification, the four samples were taken as much as 100 grams and mashed in a mixer so that the samples became more homogeneous and evenly distributed, which could later facilitate the identification process. The four samples were given the code number 1-4; the Positive control sample was given the code "P," and the negative control was given the code "N." The positive control sample used one of the red usek cracker samples which were later added with 25mg of Rhodamine B dye while the negative control sample used white usek crackers.



**Figure 1.** Usek Cracker Samples

#### **A. Testing by Wool Method**

Testing using the wool method aims to determine whether the usek cracker sample contains Rhodamine B dye or not. The principle is to separate the components of the substance by detecting the color attached to the fibers of woolen yarn. This method was chosen because it is simple, does not require sophisticated equipment, and the residue of the test result solution can be reused as a sample for KLT analysis so that one sample preparation can be used for two analyses with fairly accurate results. Tests using the wool method were carried out three times of replication to minimize the possibility of errors in the study.

The first stage carried out was a sample of 100 grams of usek crackers soaked with 150mL of 2% ammonia overnight in a beaker glass. The purpose of such soaking is to draw dye from usek crackers. Then the solution is filtered with the aim that the dye is separated from impurities that can interfere with absorbance. To speed up the dissolution of crackers usek, then the filtrate obtained in the soaking process is concentrated by heating it using a water bath.

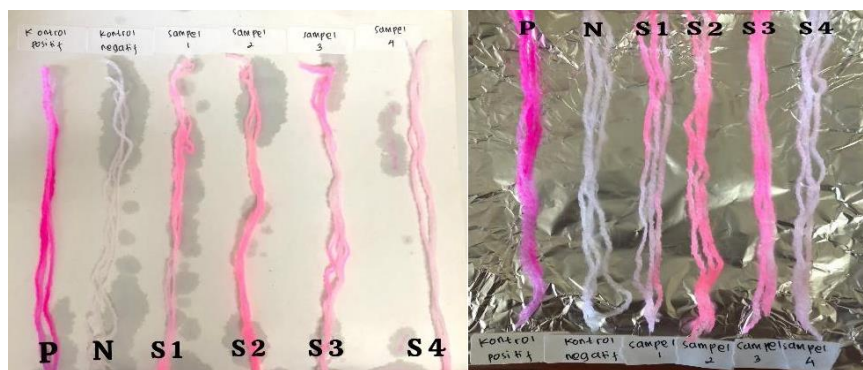
The next step is to obtain a residual solution and then dissolve it with 10mL of 10% acetic acid; this aims to prevent Rhodamine B from changing from an ionized form to a neutral form. After that, a 15cm long strand of wool thread is soaked in the solution and simmered for 10 minutes until the dye absorbs into the woolen thread. The purpose of soaking the wool thread in acetic acid solution is to extract Rhodamine B from the sample so that the color of the wool thread changes from white to bright red, indicating the presence of Rhodamine B in the sample.

Wool yarn acts as an absorbent, while acetic acid creates an acidic atmosphere that attracts Rhodamine B and produces a bright red color in wool yarn (Bai, 2022). The mechanism of absorption of Rhodamine B by wool yarn occurs due to the chemical composition of wool yarn, which consists of peptide bonds containing cystine, glutaric acid, lysine aspartic acid, and arginine (Frunza et al., 2020). Rhodamine B is able to penetrate the cuticle layer by converting cystine into an acidic form.

The breaking of the S-S bonds of cystine in an acidic atmosphere allows Rhodamine B to enter into the fibers of the wool yarn so that the color is absorbed by the wool thread. In identification using the wool method, absorbed Rhodamine B cannot be removed by washing it with water (Yugatama & Hapsari, 2021). The next step involves washing the woolen thread using a spray bottle filled with aquadest. In this method of wool, a positive result is characterized by the red color of Rhodamine B on wool threads that cannot be removed with water.

## B. Test Results with Wool Method

The results obtained by qualitative testing through the wool method on the three samples of red usek crackers with three replicated treatments can be seen in Figures 2 and 3:



**Figure 2.** Test Results of Wool Method (Replication I) and (Replication II)

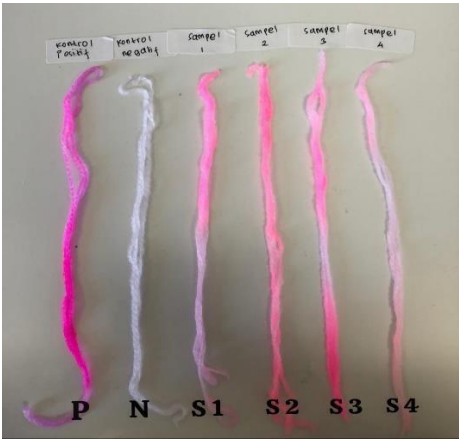


Figure 3. Test Results of Wool Method (Replication III)

Table 1. Results of Sample Analysis of Crackers by Wool Method

| Sample Code      | Replication 1                                 |        | Replication 2                                 |        | Replication 3                                 |        |
|------------------|-----------------------------------------------|--------|-----------------------------------------------|--------|-----------------------------------------------|--------|
|                  | Observation                                   | Result | Observation                                   | Result | Observation                                   | Result |
| Positive control | The color of woolen threads can not be washed | +      | The color of woolen threads can not be washed | +      | The color of woolen threads can not be washed | +      |
| Control negative | The color of woolen yarn is washable          | -      | The color of woolen yarn is washable          | -      | The color of woolen yarn is washable          | -      |
| Sample 1         | The color of woolen threads can not be washed | +      | The color of woolen threads can not be washed | +      | The color of woolen threads can not be washed | +      |
| Sample 2         | The color of woolen threads can not be washed | +      | The color of woolen threads can not be washed | +      | The color of woolen threads can not be washed | +      |
| Sample 3         | The color of woolen threads can not be washed | +      | The color of woolen threads can not be washed | +      | The color of woolen threads can not be washed | +      |
| Sample 4         | The color of woolen threads can not be washed | +      | The color of woolen threads can not be washed | +      | The color of woolen threads can not be washed | +      |

Based on the test results in Table 1. using the wool method, both on replication I, II, and III, it was seen in all samples that the color attached to the wool thread produced by usek crackers could not be washed by water. Then, when compared to the positive control and negative control, the test produced almost the same color as the negative control. These results indicate that the four samples of red usek crackers positively contain rhodamine B. In addition, wool method testing was carried out three times replication with the same results showing that the research carried out was quite accurate because the purpose of self-replication was to avoid result errors at the time of research.

The use of rhodamine B dye that is intentionally added in food products is certainly very harmful to health. The existence of some food manufacturers who still use rhodamine B in their products, especially usek crackers, can be caused by wanting to increase the quality of the usek crackers so that the color produced in the crackers is more plugged so that it can attract consumers to buy it then the price of rhodamine B is affordable and available in small packaging on the market that is easily purchased by producers. In addition, other reasons are the lack of knowledge of manufacturers about the dangers of adding rhodamine B chemicals for health and also the level of public awareness is quite low.

### **C. Testing by KLT Method**

To identify the content of Rhodamine B in a sample of usek crackers, the KLT or Thin Layer Chromatography (TLC) method can be used, which is a separation technique based on the difference in the partitioning of substances between the stationary phase and the mobile phase, based on the principle of "like dissolve like". In this principle, polar compounds will be carried by the polar mobile phase, while non-polar compounds will be carried away by the non-polar mobile phase. Along with that, a compound will be increasingly carried away by the mobile phase if its polarity is close to the eluent (Haq et al., 2023).

The KLT method was chosen because of its faster migration process, higher sensitivity compared to paper chromatography, as well as its ability to provide effective separation (Meng et al., 2023). In the analysis using the KLT method, there is a stationary phase consisting of silica gel plate GF254 and a mobile phase made of a mixture of ammoniac solvent: ethyl acetate: methanol with a ratio of (1: 15: 1). The choice of mobile phase combination is due to the polar properties of acetic acid, ammoniac, and methanol, which are expected to elute Rhodamine B effectively because Rhodamine B also has polar properties.

The polarity of a compound that is increasingly similar to the polarity of the eluent or its mobile phase makes the compound more easily eluted by the mobile phase used (Jandera & Hájek, 2018). In addition, the combination of ammoniac mobile phase: ethyl acetate: methanol used gives an optimal  $R_f$  value, which is around 0.65, which is still within the range of  $R_f$  values that are considered good, between 0.2 to 0.8 (Khasanah, Rusmalina, Safira, Setyorini, & Amanah, 2022).

The results of the color formation reaction in the previous wool yarn method were added with an alkaline solution, namely 10 ml of 10% ammonia (dissolved in 70% ethanol), for each sample, then heated using a water bath to make the results more concentrated. The result of this color formation reaction will be used as a snippet solution in the KLT method. The wool thread will give off color, and the color component will be added to the snippet solution. The principle is the heating of dyes from the sample into fat-free wool yarn in an acidic atmosphere; then the color is dissolved or dissolved by a base (Agsari & Guntarti, 2023).

After that, the sample solution that has been obtained is placed on a silica gel plate GF254 with dimensions of 10 cm long and 5 cm wide and given upper and lower limits at distances of 0.5 cm and 1 cm, respectively. The function of determining this limit is to mark the mileage of the eluent. The lower limit is set at a distance of 1 cm so that spots or stains are not submerged by the eluent. Solution placement uses capillary pipes with a distance of 1 cm between fous, which are adjusted to the width of the plates to prevent fouling that is too close together and avoid mixing spots from each sample.

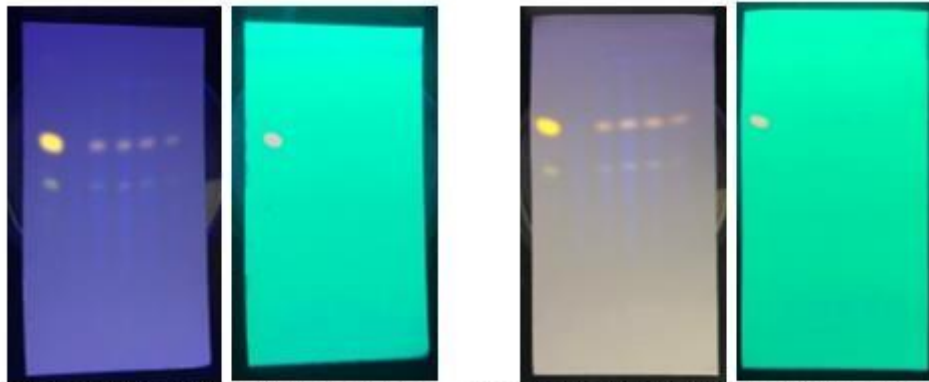
The next step involves the saturation phase using filter paper inserted into a chamber container containing the mobile or eluent phase. This saturation process aims to ensure that the steam produced from the eluent solution can be evenly distributed over the entire chamber surface so that the movement of spots can be optimal. After the eluent spreads over the entire surface of the filter paper, the filter paper is removed, and the decoded silica plate is carefully inserted into the chamber. When the eluent has reached the upper limit, the silica plate is lifted and dried in air to evaporate the remaining solvent that is still attached to the plate so that the evaporation is perfect and the resulting stain can be clearly seen (Bai, 2022).

After the plate is dried, the spots formed on the plate are observed visually and under UV light with wavelengths of 254nm and 366nm. If it looks visually pink and fluoresces yellow or orange under 366nm UV light, it indicates the presence of Rhodamine B in the usek cracker sample (Garjito, 2013). Once observed, the value of its retention factor ( $R_f$ ) is calculated with the aim of strengthening the results of sample identification and comparing its value with positive controls. Positive results are characterized by the similarity of spot color between the sample and positive control, as well as the  $R_f$  value of spotting in the sample and positive control that is equal or close, with a difference in value of less than 0.2.

#### **D. Test Results with KLT Method**

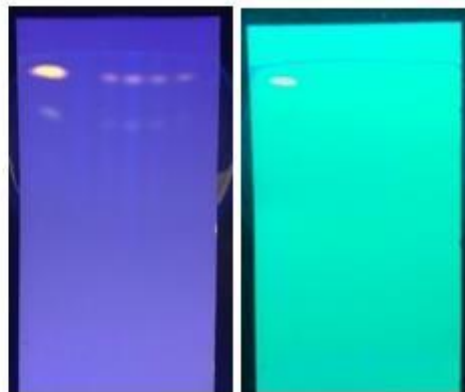
The results obtained by qualitative testing through the KLT method on the three samples of red usek crackers with three replication treatments can be seen in the picture below.

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Gambar 4. Replikasi I Uv 366nm dan 254nm

Gambar 5. Replikasi II Uv 366nm dan 254nm



Gambar 6. Replikasi III Uv 366nm dan 254nm

Table 2. Results of Cracker Sample Analysis with KLT Method

| Sample Code      | UV rays  |                     | Rf value  | Information |
|------------------|----------|---------------------|-----------|-------------|
|                  | 254 nm   | 366 nm              |           |             |
| (Replication 1)  |          |                     |           |             |
| Positive Control | Pink     | Yellow or Orange    | Rf = 0.68 | Positive    |
| Control Negative | Not pink | No Yellow or Orange | -         | Negative    |
| Sample 1         | Not pink | Yellow or Orange    | Rf = 0.66 | Positive    |
| Sample 2         | Not pink | Yellow or Orange    | Rf = 0.66 | Positive    |
| Sample 3         | Not pink | Yellow or Orange    | Rf = 0.66 | Positive    |
| Sample 4         | Not pink | Yellow or Orange    | Rf= 0.67  | Positive    |
| (Replication 2)  |          |                     |           |             |
| Positive Control | Pink     | Yellow or Orange    | Rf = 0.58 | Positive    |
| Control Negative | Not pink | No Yellow or Orange | -         | Negative    |
| Sample 1         | Not pink | Yellow or Orange    | Rf = 0.56 | Positive    |
| Sample 2         | Not pink | Yellow or Orange    | Rf = 0.57 | Positive    |
| Sample 3         | Not pink | Yellow or Orange    | Rf = 0.57 | Positive    |
| Sample 4         | Not pink | Yellow or Orange    | Rf= 0.58  | Positive    |
| (Replication 3)  |          |                     |           |             |
| Positive Control | Pink     | Yellow or Orange    | Rf = 0.72 | Positive    |
| Control Negative | Not pink | No Yellow or Orange | -         | Negative    |
| Sample 1         | Not pink | Yellow or Orange    | Rf = 0.72 | Positive    |

|          |          |                  |           |          |
|----------|----------|------------------|-----------|----------|
| Sample 2 | Not pink | Yellow or Orange | Rf = 0.72 | Positive |
| Sample 3 | Not pink | Yellow or Orange | Rf = 0.73 | Positive |
| Sample 4 | Not pink | Yellow or Orange | Rf = 0.74 | Positive |

Based on the test results using the KLT method can be seen in Table 2. that the results of the KLT plate under UV light 254 in the positive control produced a pink color, and in all four samples, there was no visible or no pink color was found. However, when irradiated under 366nm UV, both the positive control and the four samples all produced yellow or orange. These results indicate that the four samples of red usek crackers positively contain rhodamine B.

Then, for the results of positive control Rf values with samples 1 to 4 in replication I, II, and III, the values are not too different where the difference in Rf values of samples with positive controls is still in the range of  $< 0.2$ . In the positive control, consecutive results of 0.68 were obtained; 0,58, 0.72 with an average of 0.66. In sample 1, it produces an Rf value of 0.66, 0,56, 0.72 with an average of 0.64. For sample 2, the Rf value is 0.66, 0,57, 0.72 with an average of 0.64. In sample 3, it produces a value of 0.66, 0,57, 0.73 with an average of 0.65. Lastly, for sample 4 it yields an Rf value of 0.67, 0,58, and 0.74 with an average of 0.66.

From qualitative analysis using the Thin Layer Chromatography (KLT) method on 4 samples of usek crackers, it can be concluded that the results are positive for containing Rhodamine B. This is evident from the similarity or incongruity of the Rf value of the sample with a positive control. The Rf value is an indicator of the separation ability of substances using planar chromatography (TLC), where a high Rf value indicates better separation ability by eluents, while a low Rf value indicates a lower separation ability. Factors such as the chemical structure of the compound, its absorbent properties and activities, solvent cleanliness, saturation level, experimental technique, temperature, and equilibrium conditions can affect the results of separation using TLC (Sadeghi, Rajabiyani, Nabizade, Meygolinezhad, & Ahmady, 2024).

Foods containing rhodamine B are very dangerous when consumed because rhodamine B itself is a textile chemical dye, not a special dye for food. Long-term consumption of Rhodamine B in food can lead to cancer and impaired liver function. A person can experience symptoms of poisoning immediately after consuming large amounts of Rhodamine B. If exposed to Rhodamine B powder, it can result in inflammation of the eye, while if inhaled, it can cause inflammation of the respiratory tract (Salamah & Permatasari, 2023).

To prevent the dangers of Rhodamine B, people need to know the characteristics of foods containing Rhodamine B, such as uneven food color; there are several dots or lumps of color on food, brighter and shinier colors, and slightly bitter or more bitter taste when consumed (Ridjal & Kasma, 2022). With the discovery of positive usek crackers containing Rhodamine B, it is hoped that the public will be more careful in consuming colored foods, especially red usek crackers circulating in Batang Regency and its surroundings, and it is hoped that the local health office will take more

firm action on the prohibition of Rhodamine B dye in food or other hazardous materials and conduct supervision in the form of more optimal raids.

## Conclusion

Expanding upon the conclusion drawn from the tests utilizing the wool method and Thin Layer Chromatography (TLC) or KLT method, it is evident that all 4 samples of red usek crackers obtained from producers in Kasepuhan Village, Batang District, were found to contain Rhodamine B dye. The presence of Rhodamine B in these food products raises significant concerns regarding their safety for consumption. Rhodamine B is a synthetic dye that is not permitted for use in food products due to its potential health risks. Its detection in the red usek crackers indicates a serious breach of food safety regulations and underscores the need for stringent monitoring and enforcement measures to ensure the integrity of food products in the region. Furthermore, this finding highlights the importance of regular quality control checks and adherence to food safety standards by producers to safeguard public health and prevent the proliferation of harmful substances in the food supply chain. Immediate action should be taken to remove the contaminated products from the market and investigate the source of contamination to prevent similar incidents in the future.

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