Study of Rhodamine B Dyes Content in Snacks of Karuwisi Traditional Market Makassar, South Sulawesi, Indonesia

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Abstract. Snack is food that is purchased in a ready-to-consume form and is widely preferred because of its relatively cheap price, easy to obtain, attractive and varied appearance. Rhodamine B dye is one of the colouring substances prohibited for food and is declared as hazardous substance according to Regulation of Health Minister Indonesia No. 722/Menkes/Per/IX/1988 regarding dyes that are declared dangerous and prohibited in Indonesia as well as in the Decree of the Directorate General of POM No. 239/Menkes/Per/V/1985 regarding certain dye stuffs declared as hazardous materials. This study aimed to recognize the levels of Rhodamine B dye in snacks traded. This was descriptive survey research with laboratory examination by analysing Rhodamine B dye on snacks by the results of UV-Vis spectrophotometric method. Population in this study were all snacks marketed in Karuwisi Traditional Market, while the sample was nine snacks consisting of: chili sauce, layer cake, red chocolate cream, red curly crackers, cassava chips, red jelly, red popcorn, cassava crackers, and macaroni crackers because the nine samples were physically identical to Rhodamine B colouring agent, namely the use of a striking red or pink colour. The sampling technique was total sampling. The results revealed that of the nine samples, only one sample was positive for Rhodamine B, namely cassava cracker containing 7.960µg/g. It concluded that red cassava cracker was a wholesale snack at Karuwisi Traditional Market which is positive for Rhodamine B.

1. Introduction
Snack foods play an important role in providing energy and other nutrients for consumers. Globally, food-borne diseases are reported to increase significantly every year [1]. WHO data reveals that more than 50% of cases of diarrhea occurred are caused by contaminated food and lead to 550 million illnesses and 230,000 deaths [1][2]. In addition, Africa and Southeast Asia are regions that perform a high incidence and mortality rate due to food poisoning. Developing countries have a 4 times higher risk of experiencing food poisoning than developed countries. This risk is caused by low community hygiene such as inadequate water preparation, low levels of community knowledge and unsupported food safety regulations [2].

Snacks are one of the ready-to-eat foods found in traditional market and are routinely consumed by most people. According to the National Poisoning Information Center (SikerNas) of the Food and Drug Monitoring Agency of Indonesia (BPOM-RI), food poisoning in Indonesia based on case reports since 2014 tends to fluctuate. In 2014 the incidence of food poisoning was 974 cases and tended to decrease to 697 cases in the following year. Meanwhile, in 2016 food poisoning increased to 791 cases. Throughout July to September 2017, SIKerNas collected incident cases from online mass media registered with the press council. Incidents of poisoning caused by snacks of street vendors were 6 with
88 victims, packaged processed foods were 2 with 37 victims, fresh food was 1 with 7 victims and 1 of them died, and unknown cause was 1 with 7 victims and 1 of them died [3]. Data from the Food and Drug Supervisory Agency (BPOM) in 2012 in Paratmanitya and Aprillia (2016) depicted that the incidence of poisoning due to consuming food was highest position by 66.7%. One of the causes of food poisoning is the presence of chemical contaminants in food, such as borax, formalin and rhodamine. In these data, there were 2.93% of snack food samples in school children containing borax, 1.34% of formalin, and 1.02% of rhodamine-B [4].

This study aimed to recognize the levels of Rhodamine B dye in snacks at Karuwisi Traditional Market Makassar. While the specific purpose was determining the contains of Rhodamin B dye in nine samples of snacks consisted of: chili sauce, layer cake, red chocolate, red curly crackers, cassava chips, red jelly, red popcorn, cassava crackers, and macaroni crackers.

2. Materials and Methods
The type of research is a descriptive survey with laboratory tests to determine the content of Rhodamin B dye in snacks. Sample examination will be carried out at Health Laboratory Center Makassar (BBLK). The population is all snacks sold in Karuwisi Traditional Market. While the sample is nine types of snacks such as: chili sauce, layer cake, red chocolate, red curly crackers, cassava chips, red jelly, red popcorn, cassava crackers, and macaroni crackers, because all of these samples are physically identical to the dye Rhodamine B, with bright red or pink color, selected by total sampling.

The procedure for bringing samples of snacks from the market to the laboratory is as follows:

a. Initial recording of all types of sampled snacks.
b. Samples are packed in sterile and dry plastic containers (Zip Lock).
c. The container is labeled including the name of samples and codes.
d. The container is packed safely during the transportation process.
   This research procedure includes:
   a. Observation
      In this study, researchers observed using a planned procedure, namely seeing and recording the number and level of certain activities related to the problem.
   b. Documentation
      This aimed to find data concerning variables in the form of notes, transcripts, books, newspapers, magazines, agendas and so on. In this study, researchers took data through written and electronic documents using a camera.
   c. Analysis
      Coloring Rhodamine B on snacks was analyzed in UV-Vis spectrophotometry method.

To determine the content of dye, Rhodamine B color standard series was used.

a. Tools:
   1) Erlenmeyer 250 ml, 500 ml and 1000 ml
   2) 100 ml and 1000 ml beakers
   3) Glass funnel
   4) Pipette
   5) Stirring rod
   6) Stamper mortar
   7) Measuring cup
   8) Analytical balance
   9) Hot plate
   10) Fat-free wool yarn
   11) Whatman filter paper
   12) UV-Vis Spectrophotometry
b. Materials/reagents used:
   1) Acetic acid
2) Ammonia  
3) Ethanol 70%  
4) Food dye standard solution (Rhodamine B)  
5) Aquadest water  
6) Elution solution (n-butane : ethyl acetate : ammonia = 10 : 4 : 5)

Furthermore, the samples that have gone through the extraction step with standard procedures are measured by UV-Vis Spectrophotometry to assess the content of Rhodamine B. Calibration curve is applied with the regression equation $y = bx \pm a$ in order to calculate the levels of Rhodamine B.

3. Result and Discussion

There are nine types of snack samples suspected of containing Rhodamine B because of their striking reddish or pink color, namely: chili sauce, layer cake, red chocolate, red curly crackers, cassava chips, red gelatin/jelly, red popcorn, cassava crackers and macaroni crackers. The process of laboratory examination on samples took a long time at Health Laboratory Center Makassar. This is due to the large number of samples examined at Health Chemical Installation during the COVID-19 pandemic. The results of the laboratory examination were issued on November 10, 2020 with the following results:

| Samples            | Rhodamine B (µg/g) |
|--------------------|-------------------|
| Cassava crackers   | 7,960             |
| Red curly crackers | Negative          |
| Red popcorn        | Negative          |
| Red jelly          | Negative          |
| Layer cake         | Negative          |
| Cassava chips      | Negative          |
| Macaroni crackers  | Negative          |
| Chili sauce        | Negative          |
| Red chocolate      | Negative          |

The results of the laboratory examination showed that from the nine samples, there was only one positive sample containing Rhodamine B, namely cassava cracker by 7.960 µg/g.

Snack according to FAO (Food and Agriculture Organization) is food prepared and sold by street vendors on the streets and in other public crowded places which are directly eaten or consumed without further processing or preparation [5][6]. Food poisoning is the emergence of clinical symptoms of a disease or other health problems due to contaminated food. Foods that cause poisoning are usually contaminated with physical, microbial or chemical elements in dangerous doses. This condition is due
to food management that does not meet health requirements and ignore the rules of food hygiene and sanitation. There are several causes of food poisoning, namely [7]:

a. Natural food ingredients, namely foods that naturally contain toxins, such as poison mushrooms, puffer fish, green cassava, poison yam.

b. Microbial infection, which is caused by bacteria in the digestive tract of food that enter the body or ingestion of microbes in large numbers, which then live and multiply, such as *Salmonellosis* and *Streptococcus*.

c. Microbial toxins, namely toxins produced by microbes in food that enter the body in dangerous amounts.

d. Chemicals, namely hazardous materials in food that enter the body in dangerous amounts, such as arsenic, antimony, cadmium, pesticides with symptoms of respiratory depression to coma or die.

e. Allergies, namely allergens in food that cause sensitive reactions to susceptible people, such as histamine in shrimp, tuna, cooking spices and so on.

The use of synthetic dyes in food, although it has a positive impact on producers and consumers, including making a food more attractive, evens out the color of food, and restores the color of the basic ingredients that are lost or changed during processing, but it may also damage human health.

Rhodamine B is a synthetic dye in the form of a crystalline powder, green or reddish purple in color, odorless, and a bluish red/strong fluorescence solution in water and comes from the cationic dyes group. Rhodamin B is widely used as a dye for paper, textiles, wool, silk and as a reagent for the analysis of antimony, cobalt, bismuth and others [8].

a. Symptoms when exposed to Rhodamine B:
   1) If swallowed, it may cause irritation to the digestive tract and symptoms of poisoning and red or pink urine.
   2) If in contact with skin, it may cause irritation to the skin.
   3) If in contact with eyes, it may cause eye irritation, redness, or edema of the eyelids.
   4) If inhaled, it may cause irritation to the respiratory tract.

b. The characteristics of foods containing Rhodamine B:
   1) The color looks bright (colorful).
   2) There is a slight bitter taste (especially in syrup or lemonade).
   3) There is itching in the throat after consuming it.
   4) The smell is not natural as food.

c. Actions when exposed to Rhodamine B:
   1) If exposed to skin, remove clothing, jewelry, or shoes contaminated with Rhodamine B. Wash skin with soap and running water until clean for approximately 15 to 20 minutes, if necessary, contact a doctor.
   2) In case of contact with eyes, rinse with running water or physiological saline solution, blinking the eyes until it is confirmed that the remaining Rhodamine B is no longer present/clean, if necessary, consult a doctor.
   3) If swallowed and vomiting occurs, place the head lower than the hips to prevent vomiting entering the respiratory tract.
   4) If the victim is unconscious, tilt the head to the side or to one side.

The government has issued a regulation regarding the violation of the use of Rhodamine B in medicine, food and cosmetics through the Minister of Health Regulation No. 239/MenKes/Per/V/Year 1985 concerning Certain Dyes Declared as Hazardous Substances. In addition, specifically for food, Ministerial Regulation No. 722/MenKes/Per/IX/Year 1988 concerning Food Additives stipulates that these compounds may not be used as food coloring. For food coloring, it is recommended to use natural or synthetic dyes based on Ministerial Regulation No. 722/MenKes/Per/IX/Year 1988 concerning Food Additives [8].

The Food and Drug Supervisory Agency (BPOM) has socialized the public to be smart in choosing food. By knowing the characteristics of food with Rhodamine B, it is hoped that people should aware in choosing foods that are safe for their health. Foods containing Rhodamine B will have a striking red/pink
color, uneven coloration, and slightly bitter taste if consumed. Foods that have certification from BPOM will be guaranteed not to contain harmful ingredients, while products containing Rhodamine B will not include codes, labels, brands, or distribution permits from BPOM [9].

Rhodamine B compounds show both acute and chronic effects if ingested. In acute conditions, it can be irritating to the digestive tract and may cause toxic effects. In addition, exposure from vegetable dyes containing Rhodamine B in excess may lead to red or pink urine. While the chronic impact, if Rhodamine B is widely absorbed by the digestive tract, it will show strong protein binding. High concentrations in food if given to rats can cause liver damage [8].

Crackers are one of the favorite foods by many people, while producers often add various kinds of colors to crackers, especially red in order to attract attention. The dyes applied may come from natural and synthetic dyes, but there are still manufacturers which use prohibited synthetic dyes.

Identification of Rhodamine B in crackers in North Jakarta Market also showed that of the 24 samples of crackers identified, there were two samples that were positively identified using Rhodamine B dye. Onion cracker Cap Kambing Kembar from Sunter Podomoro Traditional Market and onion cracker (large curly round) from Warakas Traditional Market because visually they show a pink stain and fluoresces under UV light 254nm and its Rf value is same as the comparison standard for Rhodamine B, each of which is 0.8 for eluent I and 0.89 for eluent II. Therefore, the sample was declared positive for containing synthetic dye Rhodamine B [10].

Hedie and jusnita (2017) stated that the use of these prohibited compounds by producers is due to the price being more affordable, attractive and durable compared to natural dyes. In addition, the color appearance produced by natural dyes is very different from synthetic dyes which is very prominent and attracts consumers, while natural dyes produce a pale red so that consumers are less interested, and difficult to obtain. In contrast to synthetic dyes, many are found on the market at affordable prices and their use is quite practical [10].

Widaryanto (2018) also declared that the lack of knowledge about the dangers of synthetic dye additives in food by producers is a factor that is often found in the field. Of the 10 samples of colored crackers studied, there were six samples with negative results (-) which were indicated by a clear color after passing the chromatographic test, while the other four samples showed positive results (+) which were indicated by pink color. This is because most of the cracker producers in Tanjung Anyar Traditional Market Mojokerto, have switched to using permitted food colorings such as anthocyanin, curcuma, carmine, caramel, erythrosine, and tartrazine [11].

The content of Rhodamin B in cassava crackers has also been found in Malang City Traditional Markets. There are 6 samples of cassava crackers, 2 samples from each of 3 markets, namely Besar Market, Sukun Market and Kedungkandang Market. The results revealed that 2 samples were positive contained Rhodamine B [12]. Similarly, red crackers at Antasari Traditional Market Banjarmasin, found 1 out of 5 samples (korek api cassava crackers) [13]. Otherwise, different results were shown in samples of prawn crackers at Masomba Traditional Market in Palu, which were safely to consume because all the crackers sold did not contain Rhodamin B [14].

Therefore, consumers who have the right to food safety should know the dangers of food products contaminated with toxic hazardous materials. Zazili and Hartono (2016) have implemented a consumer empowerment model against the dangers of food products contaminated with chemical additives through direct counseling to the public using lectures, discussions, and audio-visual presentations [15].

The information submitted is in the form of ways to identify food products that are indicated to be contaminated or use food additives that are prohibited such as Formalin, Borax, Rhodamin B, and Metanil Yellow. In order to increase legal knowledge, knowledge of consumer rights is regulated and guaranteed by Law Number 8 of 1999 concerning Consumer Protection. It is essential to convey information regarding consumer rights so that consumers are able to know and understand that the act of using prohibited food additives is an act that violates the law, and then consumers may sue business actors who violate consumer rights. Awareness, legal compliance and consumer behavior also determine the practice of law protection and enforcement [15].
4. Conclusions
It concluded that red cassava crackers are snacks sold at Karuwisi Traditional Market Makassar which are positive for Rhodamin B dye. It is encouraged to consumers to aware of the dangers of red snacks which may contain Rhodamine B. Moreover, traders should not use Rhodamine B as a food additive because of the numerous acute and chronic impacts that can damage health. In addition, BPOM should periodically carry out inspections to snacks or foods suspected containing hazardous materials in traditional markets.

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