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ANALYSIS OF RHODAMIN B AND METHANYL YELLOW ON CANDY FRUIT CIRCULATED IN THE CITY OF BANJARMASIN USING HPLC METHOD

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ABSTRACT

Candied fruit is one type of food that many people like, both in terms of color, smell, taste and texture. Consumers need to be careful in consuming candied fruit that has color, because there are still many fruits that use prohibited dyes, especially Rhodamin B and Methanyl Yellow. The content of Rhodamine B and Methanyl Yellow in candied fruit causes the color of candied fruit to become more striking. Rhodamine B and Methanyl Yellow when taken for a long time can cause liver function disorders and symptoms of acute poisoning. The purpose of this study was to determine the presence or absence of rhodamine B and methanyl yellow dyes in candied fruit, to determine the amount of rhodamine B and methanyl yellow dyes to candied fruit and to determine the effect of sampling locations on candied fruit in five districts of the city of Banjarmasin. The research design was analytic observational with a cross sectional design. The method used was HPLC. The population and samples were candied fruit located in 5 sub-districts of Banjarmasin which were taken by purposive sampling technique. The results obtained in candied fruit circulating in the city of Banjarmasin that there is no rhodamine B and methanyl yellow dyes and the levels of rhodamine B and methanyl yellow are 0. Based on data analysis, there is no effect of sampling location on candied fruit in five districts of Banjarmasin city.

Keywords: Candied Fruit, HPLC, Rhodamine B, Methanyl Yellow

1. Introduction

Food is something that comes from biological and air sources, both processed and unprocessed, which is needed for human consumption, including food additives, food raw materials and other materials used in the process of preparing, processing, making food or beverages. Food Quality according to Government Regulation Number 86 of 2019 Article 1 concerning Food Safety is the value determined on the criteria for safety and food nutrition content (Pemerintah et al., 2019).

Foods that are liked by many people are foods that look attractive both in terms of color, smell, taste and texture that are better. Synthetic dyes that are often used in food additives are rhodamine B, methanyl yellow, ponceau and tartrazine. Opportunities that occur compared to

rhodamine B and methanyl yellow dyes in food can occur because the price is cheap, easy to obtain and this food coloring agent has a more attractive color with natural dyes for food. The use of rhodamine B and methanyl yellow textile dyes in food is strictly prohibited because they can accumulate in the human body and are carcinogenic which in the long term causes abnormalities in body organs (Masthura, 2019).

Based on the Regulation of the Minister of Health of the Republic of Indonesia No. 722/MenKes/Per/IX/88, rhodamine B is a dye in the form of a crystalline powder, green or reddish purple in color, odorless, soluble in water, with a strong bluish red color. Rhodamine B dye is prohibited from being used as a food additive because it is harmful to the human body. The use of rhodamine B in food for a long time (chronic) can lead to impaired liver function and cancer. However, if exposed to large amounts of rhodamine B, in a short time, acute symptoms of rhodamine B poisoning will occur (Rahayu & Mahmuda, 2016).

Methanyl yellow is a synthetic dye in powder form, brownish yellow in color, soluble in water and alcohol, slightly soluble in benzene and ether, and slightly soluble in acetone. These dyes are commonly used as dyes in textiles, paper, inks, plastics, leather and paints, and as acid-base indicators in laboratories. However, in Indonesia this dye is often misused to color various types of food, including crackers, noodles, tofu, candied fruit and yellow snacks. The use of methanyl yellow in food can cause irritation to the respiratory tract, skin irritation, eye irritation and the danger of bladder cancer. If ingested, it can cause nausea, vomiting, stomach pain, diarrhea, heat, discomfort and low blood pressure, which is a further danger of causing bladder cancer (BPOM, 2015)

According to a previous study by Dewi Ratna on the effect of rhodamine B on the cerebellum and brain stem tissue, Rattus norvegicus had direct and indirect effects on the expression of BAX and BCL-2 in the cerebellum and cerebellum tissue in Wistar rattus norvegicus by showing significant results ($p < 0$,05). Based on these results, it was concluded that rhodamine B as a food additive has toxic properties (Sulistina & Martini, 2020). In methanyl yellow according to research by Ishfaq Shafi Khan on the genotoxic effect of two food colorings methanyl yellow and carmoisine, methanyl yellow has a cytotoxic effect on Allium Cepa L with the occurrence of chromosomal aberrations, namely anaphase stickiness and metaphase stickiness caused by inhibition of several proteins (Shafi et al., 2020)Meanwhile, according to Abdulrahman L Al-Malki's research on the weakening of bee honey from the induction of hepatotoxic methanyl yellow in rats, liver damage caused cytosolic leakage by increasing levels of hepatospecific enzymes in serum (Al-malki et al., 2013)

Candied fruit sold in the Banjarmasin area, some have color and some do not. Candied fruit is preserved using sugar because sugar has a high level so candied fruit can avoid growth microorganisms. Candied fruit that has too bright color is very susceptible to the use of rhodamine B and methanyl yellow dyes.

Based on this description, this study aims to determine whether the processed fruit products contain rhodamine B and methanyl yellow dyes, as well as determine the amount of concentration and determine the influence of the sampling location on the levels of rhodamine B and methanyl yellow obtained in candied fruit in the Banjarmasin area. North, Central Banjarmasin, South Banjarmasin, East Banjarmasin and West Banjarmasin.

2. Material and Methods

The research method used High Performance Liquid Chromatography (HPLC). The materials used in this study were aquabidest, 10% ammonia solution (NH_4OH), concentrated hydrochloric acid (HCl), concentrated sulfuric acid (H_2SO_4), 10% sodium hydroxide (NaOH), methanol pa (CH_3OH), acetate buffer pH 6 p.a, synthetic dyes rhodamine B ($C_{28}H_{31}ClN_2O_3$) and methanyl yellow ($C_{18}H_{14}N_3NaO_3S$) as standard.

The equipment used in this study was a set of HPLC tools, UV-Vis spectrophotometer, hot plate, blender, filter paper, parchment paper, funnel, horn spoon, stirring rod, analytical balance, volume pipette, dropper pipette, injection syringe, 50 ml beaker glass. , 100 ml, 10 ml volumetric flask, 100 ml, 100 ml measuring cup, 250 ml Erlenmayer and 0.45 μL millipore filter.

Implementation Stage

a. Qualitative work procedures

1) Sample making

a) Take a sample of candied fruit puree using a blender and weigh 25 grams put it in an Erlenmeyer

b) Then add 100 ml of 2% ammonia solution in 70% ethanol and put it in an erlenmeyer containing the sample by soaking it overnight until all the dye is dissolved.

c) A colored solution that has been allowed to stand overnight and then filtered using filter paper

d) Then after being filtered the solution was transferred to a glass beaker and evaporated using a hot plate for 4 hours at a temperature of 650C, the sample became concentrated during the evaporation process.

e) Then the sample that has been vaporized is filtered using a 0.45 L syringe filter and ultrasonically

f) The sample is ready to be used for analysis in HPLC and color reaction

2) Preparation of standard solutions of rhodamine B and methanyl yellow

a) Make a standard solution of 1000 ppm by weighing 100 mg of rhodamine B and methanyl yellow dyes then put each dye into a 100 ml volumetric flask

b) Then add the aquabidest solution until the boundary marks on each volumetric flask that has been added to the dye until homogeneous

c)Then a standard solution of 10 ppm was made by pipetting 1 ml of 1000 ppm rhodamine B and methanyl yellow mother liquor then put into a 100 ml volumetric flask, add aquabidest to the mark and homogenized.

d) Standard solutions of Rhodamine B and Methanyl yellow with a concentration of 30 ppm were measured for absorption at a wavelength of 400-750 nm using a UV-Vis spectrophotometer, then the maximum wavelength was determined for analysis.

3) Determination of the optimum conditions for determining the color of methanyl yellow

a)PH buffer acetate

1. Methanyl yellow standard solution mixture was injected as much as 20 L into the column
2. Then use the mobile phase mixture of methanol:acetate buffer pH 6.0 with a ratio of 80:20 mobile phase composition then record the retention time (tR).

b) Mobile phase

1. Methanyl yellow standard solution mixture was injected as much as 20 L into the column.
2. Then use the mobile phase methanol:acetate buffer optimum pH with a ratio of 80:20 record the best separation based on retention time (tR).

4) Preparation of standard solution for determination of linear regression curve from methanyl yellow standard solution

a) Methanyl yellow standard solution of 1000 ppm was made with concentrations of 25 ppm, 50 ppm, 75 ppm, 100 ppm and 125 ppm in a 10 ml volumetric flask

b) Then inject 20 L of the solution into the column using the predetermined optimum conditions. The calibration curve is based on the concentration and area of the resulting peak

5) Preparation of 10% ammonia solution (NH_4OH)

a)Pipette 10 ml of 25% ammonia solution using a volume pipette and then put it into a 25 ml volumetric flask

b) Then add it using aquabidest until the mark line

c)10% ammonia solution ready to use

6) Test the reaction for the presence of Rhodamine B

a)Reaction with concentrated sulfuric acid (H_2SO_4)

1. Put 1 ml of the sample into a test tube by adding 3 drops of concentrated sulfuric acid (H_2SO_4)
2. Then shake, observe the changes that occur and compare with the standard solution whether it changes to a purplish red color

b) Reaction with concentrated hydrochloric acid (HCl)

1. Put 1 ml of the sample into a test tube by adding 3 drops of concentrated hydrochloric acid (HCl)
2. Then shake, observe the changes that occur and compare with the standard solution whether it changes to a purplish red color

c) Reaction with 10% ammonia solution (NH_4OH)

1. Put 1 ml of the sample into a test tube by adding 3 drops of 10% ammonia (NH_4OH)
2. Then shake, observe the changes that occur and compare with the standard solution whether it changes to a purplish red color

d) Reaction with 10% sodium hydroxide solution (NaOH)

1. Put 1 ml of the sample into a test tube by adding 10% sodium hydroxide (NaOH) 3 drops
2. Then shake, observe the changes that occur and compare with the standard solution whether it changes to a purplish red color

7) Color testing on samples using HPLC

a) A total of 20 L of sample is injected into the column

b) Then record the retention time of the peaks produced by the sample, if these peaks have a retention time that is approximately the same as the retention time of the peaks of the standard solution, it can be concluded that the sample contains these substances.

b. Quantitative work procedures

1) Testing the levels of Methanyl yellow using HPLC

a) The standard solution that has been filtered using 0.45 m millipore is taken as much as 20 L of methanyl yellow

b) Then it is injected into the column and record the resulting peak retention time

2) Testing the levels of candied methanyl yellow fruit samples using HPLC

a) Samples that have been filtered using 0.45 m millipore are taken as much as 20 L

b) Then record the retention time of the peaks produced by the sample, if these peaks have a retention time that is approximately the same as the retention time of the peaks of the standard solution, it can be concluded that the sample contains these substances.

3. Result and Disscusion

The tests carried out in this study used 5 samples of candied fruit with purchases at different places. This sample was obtained in 5 sub-districts of the city of Banjarmasin. To facilitate the identification of the research, the candied fruit samples were coded, namely samples A (Central Banjarmasin), B (East Banjarmasin), C (North Banjarmasin), D (South Banjarmasin) and E (West Banjarmasin).

The initial testing process is carried out by making sample preparations, namely by smoothing the sample using a blender available in the laboratory. The mashed sample was then added to 100 ml of 2% ammonia solution in 70% ethanol and then left overnight until the color dissolved. The purpose of adding 2% ammonia solution in 70% ethanol is because 2% ammonia can provide an alkaline atmosphere so that basic compounds will be extracted or dissolved into organic solvents. The candied fruit that has been soaked overnight is then filtered using filter paper and heated on a hotplate at 65°C for 4 hours after which it is filtered again with a 0.45 L syringe filter and the sample can be analyzed for HPLC and color reactions. Preparation of a standard solution of methanyl yellow from 1000 ppm mother liquor was made with concentrations of 25 ppm, 50 ppm, 75 ppm, 100 ppm and 125 ppm which were then filtered using a 0.45 L syringe filter for the purpose of filtering so that there were no small particles in the methanyl yellow standard solution.

A. Qualitative test

In the qualitative test used color reagents, namely concentrated hydrochloric acid (HCl), concentrated sulfuric acid (H_2SO_4), ammonia 10% (NH_4OH), sodium hydroxide (NaOH) did not form a color change to purplish red so that in samples A, B, C, D and E were declared not to contain rhodamine B.

Table 1. Results of rhodamine B dye in candied fruit samples using a color reaction

No	Sample	Rhodamine B reagent	Result
1	A	HCl, H_2SO_4, NH_4OH, NaOH	Negative
2	B	HCl, H_2SO_4, NH_4OH, NaOH	Negative
3	C	HCl, H_2SO_4, NH_4OH, NaOH	Negative
4	D	HCl, H_2SO_4, NH_4OH, NaOH	Negative
5	E	HCl, H_2SO_4, NH_4OH, NaOH	Negative

The results of the qualitative rhodamine B test carried out in table 1 samples of candied fruit circulating in the city of Banjarmasin namely samples A, B, C, D and E with 4 reagents used showed negative results where on the 5th The candied fruit sample was not detected to contain synthetic dye rhodamine B because in the color reaction test there was no change in color to purplish red and there was no withdrawal of rhodamine B dye from the ether phase to an acidic environment to obtain positive color identification results containing rhodamine B. The following is a chromatogram of a standard solution of methanyl yellow.

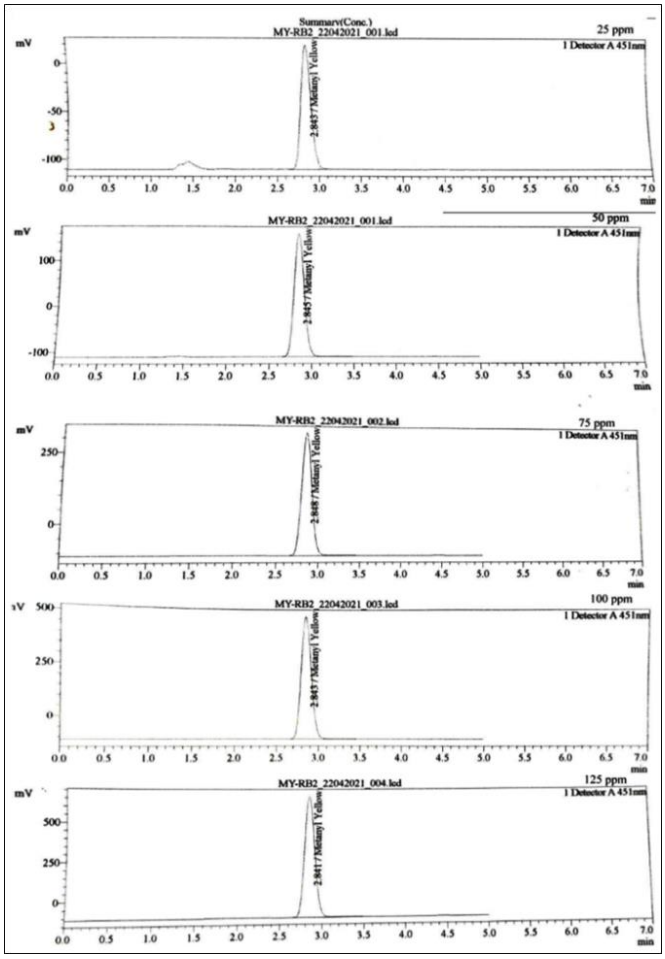

Figure 1. Chromatogram of standard solution of methanyl yellow with concentrations of 25 ppm, 50 ppm, 75 ppm, 100 ppm and 125 ppm

In the standard solution of methanyl yellow with concentrations of 25 ppm, 50 ppm, 75 ppm, 100 ppm and 125 ppm, the retention time that appears is at 2.84 minutes with the time span used in HPLC is 1 to 5 minutes. The purpose of making standard solutions with several concentrations is to determine the relationship between the concentration of the solution and its absorbance value. In the measurement of the methanyl yellow test on several samples, the following results were obtained.

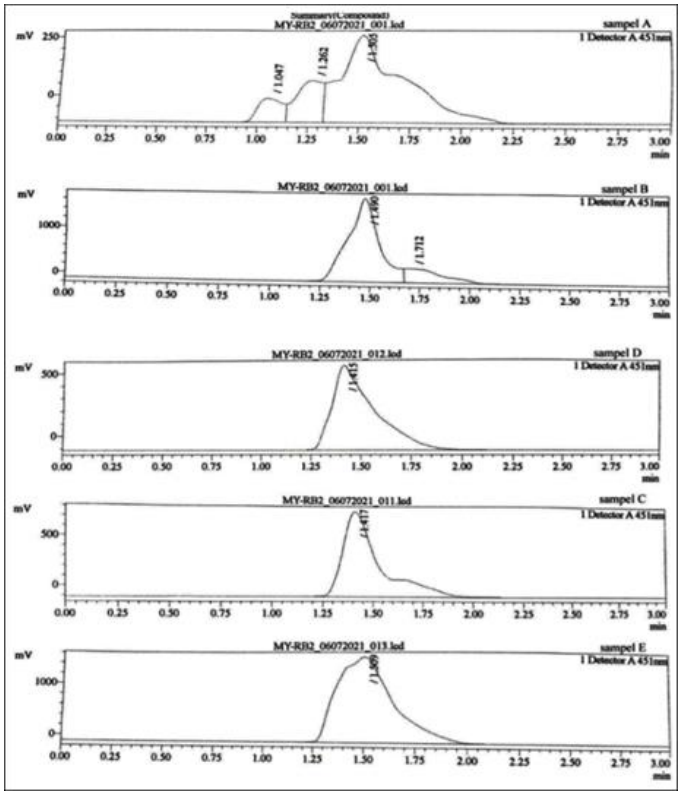

Figure 2. Chromatogranate samples A (Central Banjarmasin), B (East Banjarmasin), C (North Banjarmasin), D (South Banjarmasin) and E (West Banjarmasin)

The results of the qualitative methanyl yellow test using the HPLC test show in Figure 18, Figure 2 which were carried out on samples of candied fruit circulating in the city of Banjarmasin, namely samples A, B, C, D and E showed negative results, which means none of the samples contained methanyl yellow. This was shown because there was no chromatogram that appeared at the retention time of 2.84 minutes. The cause of the negative results was because the methanyl yellow dye was not present in the candied fruit that had been tested, but at the retention time of 1.5 minutes there were other components that had not been found. known outside of the substance under study. The negative results can also be said because methanyl yellow dye is not used in candied fruit or the dye used in candied fruit in the form of other synthetic dyes outside of the tested dyes, as well as the increased awareness system of sellers about the dangers of rhodamine B and methanyl yellow in food so that traders do not use synthetic dyes which are prohibited by the government.

B. Quantitative test

Determination of the maximum standard wavelength for synthetic methanyl yellow dye was carried out using a UV-VIS spectrophotometer at a wavelength of 400-750 nm. This is done because methanyl yellow is a colored solution that is red. Visible light has a wavelength of 400-750 nm. The dye (methanyl yellow) with a concentration of 10 ppm was measured using UV-VIS so that the max of methanyl yellow was 541 nm (Figure. 3).

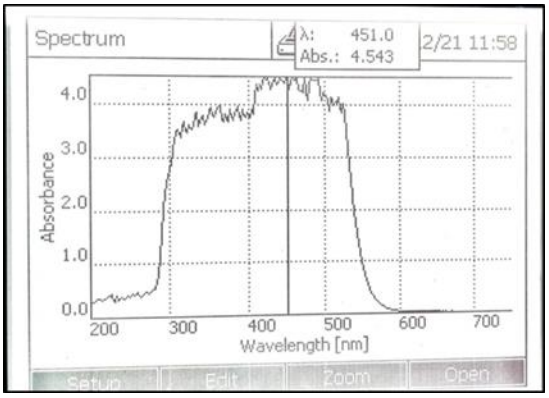

Figure 3. Spectogram of Methanyl yellow 10 ppm

The results of the measurement of the wavelength of 10 ppm obtained a maximum wavelength of 451 nm. The results of wavelength measurements using standard solutions were then made with several concentrations, namely 25 ppm, 50 ppm, 75 ppm, 100 ppm and 125 ppm. The following are the results of the quantitative test for methanyl yellow levels using HPLC on the five samples of candied fruit circulating in Banjarmasin.

Table 2. Results of quantitative methanyl yellow test using HPLC

No	Sampel	kadar *methanyl yellow* (ppm)
1	A	0
2	B	0
3	C	0
4	D	0
5	E	0

Based on the results of quantitative analysis using HPLC where there was no methanyl yellow content in 5 samples and indicated by the methanyl yellow content obtained, namely 0 where the retention time of the sample was not at the same time as the standard solution of methanyl yellow, which was 2.84 minutes. Meanwhile, The results of the analysis of rhodamine B on the measurement of levels of rhodamine B compounds using HPLC could not be carried out because the raw material for rhodamine B used was technical rhodamine B so that at the time of injection no chromatograms appeare. Factors affecting rhodamine B are not read in HPLC because the rhodamine B used is a technical rhodamine B whose purity is known to be unknown because the rhodamine B packaging does not have a purity code, such as methanyl yellow which has a purity code Pcode: 102267379.

4. Conclusion

Based on the research that there is no rhodamine B and methanyl yellow compounds in candied fruit, so it can be said that there is no effect of sampling location on the levels of rhodamine B and methanyl yellow obtained in candied fruit in the areas of North Banjarmasin, Central Banjarmasin, South Banjarmasin, East Banjarmasin and West Banjarmasin, due to the absence of rhodamine B and methanyl yellow dyes in candied fruit.

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Declaration of Interest Statement

The authors declare that they have no conflict of interests.

References

Al-malki, A. L., Amir, A., & Sayed, R. (2013). *Bees ' Honey Attenuation of Metanil-Yellow-Induced Hepatotoxicity in Rats*. *2013*.

BPOM. (2015). *Pengetahuan Bahan Berbahaya*. *978-979-37–7*, 25.

Masthura. (2019). *Identifikasi Rhodamin B Dan Methanyl Yellow Pada Manisan Buah yang Beredar Di Kota Banda Aceh Secara Kualitatif*. *1*(1), 39–44.

Pemerintah, P., Pangan, K., Jenis, D., & Tambahan, B. (2019). *Lampiran Peraturan Pemerintah Republik Indonesia*. *70*(019429), 19429–19456.

Rahayu, M., & Mahmuda, Y. I. (2016). Identifikasi Zat Pewarna Rhodamin B Dan Methanyl Yellow Pada Kerupuk Yang Dijual Di Pasar Beringharjo Yogyakarta Tahun 2016. *Jurnal Teknologi Laboratorium*, *5*(2), 3–6.

Shafi, I., Ali, N., Hamid, R., & Ahmad, S. (2020). Genotoxic effect of two commonly used food dyes metanil yellow and carmoisine using Allium cepa L . as indicator. *Toxicology Reports*, *7*(December 2018), 370–375. https://doi.org/10.1016/j.toxrep.2020.02.009

Sulistina, D. R., & Martini, S. (2020). *The effect of Rhodamine B on the cerebellum and brainstem tissue of Rattus norvegicus*. *9*, 101–104.