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## Stability of Cassava-Based Angkak Pigment in Different Extreme Conditions.

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### ABSTRACT

Angkak pigment as a natural colorant was produced from a secondary metabolite of *Monascus purpureus*. In application, this colorant was affected by physical and chemical factors. This research goal was to observe angkak pigment from cassava-based related to its solubility in water, effect of heating period, temperature and pH. Characterization analysis and stability of angkak from cassava-based showed an increasing solubility in water along with increasing water temperature up to 100°C. However, it also can be observed that heating angkak in boiled water for one hour and heating treatment above 100°C can decrease color intensity. Further, decreasing color intensity was also happened when angkak was stored in pH 4 and pH 9.

**Keywords:** angkak, stability, pigment

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## INTRODUCTION

Angkak pigment is natural colorants produced from secondary metabolite of *Monascus purpureus*. This mold was growth during fermentation of starch contained substrates such as rice, sweet potato, cassava and yam [1]. During fermentation process, color of substrate will change from its initial based on its pigment such as Monascin and Angkavilavin give yellow color while Rubropunctatin and Monascorubrin are orange and red for Monascorubramin and Rubropunctamin [2]. Based on previous research by [1], it was found that cassava is a substrate that produced the highest pigment compared to sweet potato and yam.

Angkak can be used as food colorant for yogurt, meat, sausage and also to preserve fruit, vegetables and fisheries products. *Monascus* pigment can be applied on beauty product such as lipstick, whitening or skin protection product even for silk colorant. Further, angkak can be used also as the medicine such as infectious diseases, diarrhea, dengue fever, controlling cholesterol level, HDL-cholesterol, triglyceride in blood since it contains monacolin-K [3].

As explained by [4], the stability of angkak from waste water and onggok from tapioca production is affected by physical and chemical factors such as temperature, heating period, sunlight, pH, reductor and oxidator also its solubility in water. Further, [5] noted that the benefit of *Monascus* pigment in food and beverages colorant with neutral pH, without long heating period, indirect sunlight exposure during storage and transportation. Direct sunlight exposure caused pigment degradation. Thus, this study aimed to observe cassava-based angkak stability with different physical and chemical factors.

## METHODS

### Materials

There were PDA, saline solution, cassava (Adira2 variety, Padang Pariaman), aqua dest, methanol, buffer Na-citrate, Na-phosphate, Na-carbonate used in this study. Further, spectrophotometer, haemocytometer, cabinet dryer, oven, analytical balance, pH meter, thermometer and glass equipments were also used.

### Angkak pigment production from cassava [1]

Angkak pigment from cassava was produced and characterized for its color pigment.

### Preparing material for substrate fermentation

Cassava was separated from its skin then cut into 1 cm<sup>2</sup> size with  $\pm$  50% of water content. Next, cassava was sterilized and ready to use as fermentation substrate after cooled down.

### Culture Preparation

Pure isolates of *Monascus purpureus* were inoculated into slanted PDA agar and incubated for 23 days. Five milliliters of saline solution were added to agar surfaces in order to obtain ascospores or conidia. Ascospores was scratched then suspended in saline solution. The number of spores were counted using hemacytometer then culture can be used.

### Angkak Production

Fermentation was conducted at room temperature (29-30°C) using 10% of inoculum with 50% of water content for 21 days. Each treatment was triplicated. At the end of fermentation, product was dried at temperature 40-45°C for 72 hours to obtain 7-8% of water content. Then, angkak was powdered and analysed.

## Characterization of Angkak Pigment

Physical and chemical characterization of natural pigment stability was conducted based on [4] method as follow:

### Pigment Solubility

Ten milliliters of water with different temperature which were 40°C, 60°C, 80°C and 100°C were filled to test tubes. In each test tube, about 60 mg of angkak was added and put in a vortex for 30 seconds. Next, the solution was filtered using filter paper and the absorbance of its filtrate was observed using spectrophotometer at 400, 470 and 500 nm.

### Analysis on heating period ( at temperature 100°C for 1, 2 and 3 hours)

As much as 60 mg of angkak was diluted in 100 mL of water and stirred for one minute. 20 ml of the solution then was filtered and filtrate was moved to for different test tube and boiled for 1, 2 and 3 hours. Further, absorbance of each heating treatment was observed using spectrophotometer at 400, 470, and 500 nm [6].

### Analysis of different temperature (100, 125, 150, 175 and 200°C for 1 hours)

As much as 40 mg of angkak powder were diluted in 100 mL of water and stirred for one minute. Solution was filtered then filtrate were moved to 5 different test tube with 15 mL of filtrate for each test tube. Each test tube was heated on oven with different temperatures which were 100°C, 125°C, 150°C, 175°C and 200°C for one hour. Next, absorbance of each filtrate was measured using spectrophotometer at 400, 470 and 500 nm.

### pH Analysis

A 600 mg of angkak powder was diluted in 100 mL of water and stirred for one minute. The solution was filtered and moved to two test tubes with 1 mL of filtrate for each. Then, 9 mL of buffer Na-citrate were added into filtrate to make pH 4 while other two test tubes were added by 1 mL of Na-phosphate buffer for pH 7 and 9 mL of Na-phosphate buffer for pH 9. Pigment intensity on each pH condition was observed using spectrophotometer at 400, 470 and 500 nm every 3 hours.

## RESULTS AND DISCUSSION

### Water Solubility

The result of water solubility analysis on pigment of cassava-based angkak in different temperatures are shown in Figure 1 as follow.

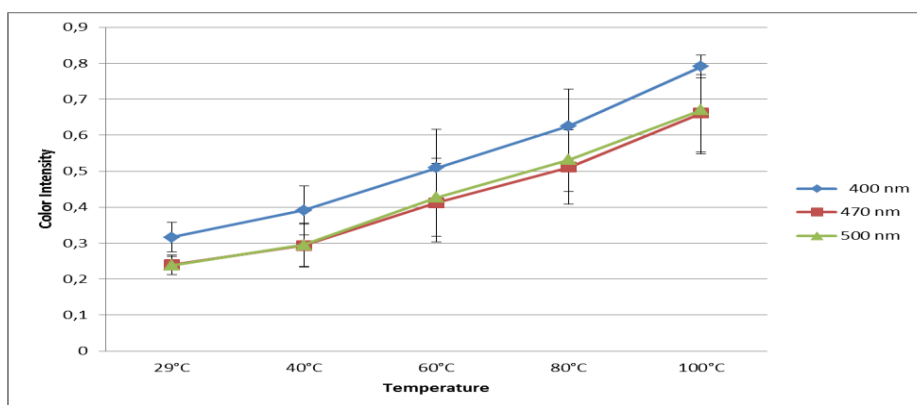
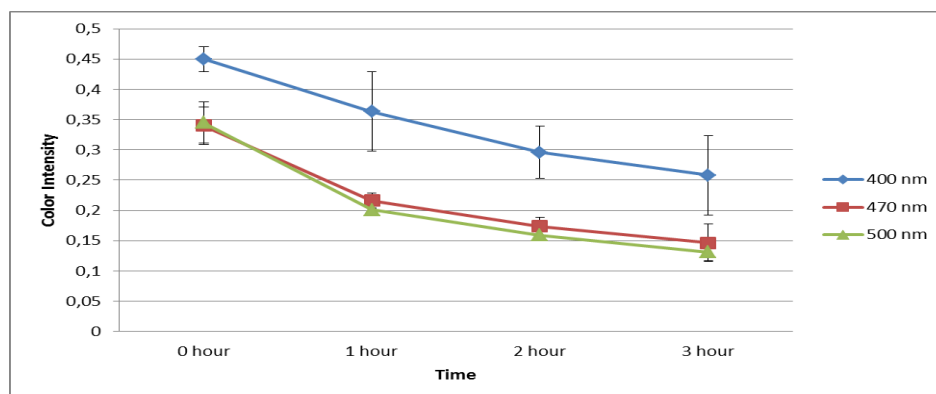


Figure 1. Pigment Solubility of Cassava-based Angkak in Water with Different Temperature Condition.

It can be observed the effect of different temperature on pigment solubility on Fig. 1. There was a trend on angkak pigment (yellow, orange and red) to dissolve easier in higher temperature up to 100°C. Moreover, the higher color intensity was also observed along with the higher temperature condition for dissolving pigment in water. Solubility at temperature 100°C resulted the highest color intensity in 400, 470 and 500 nm while the lowest color intensity was on dissolving pigment at water temperature 29°C.

### Effect of Heating Period

The results of heating period effect to color intensity of angkak pigment from cassava are shown in Figure 2 as followed.

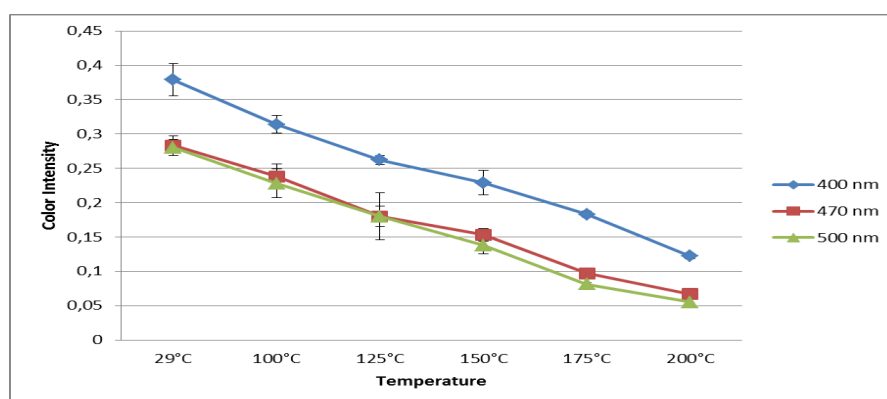


**Figure 2. Heating Period Effect to Color Intensity on Cassava-based Angkak Pigment**

Figure 2 shows the decreasing color intensity of cassava-based angkak during water boiling period (at temperature 100°C). This condition indicated defect on natural pigment along with heating at temperature 100°C. Initially, it showed a high color intensity at 100°C on 0 hours therefore color intensity was decreased significantly after one-hour heating. A longer heating period made a higher impact on color intensity decreasing indicated decaying pigment. This condition can be found on three color pigment of cassava-based angkak (yellow, orange and red)

### Effect of Temperature

The results of analysis of heating temperature on the cassava-based angkak pigment in water to color intensity are shown on Figure 3. It can be observed from Fig. 3 that temperature had significant effect to pigment stability. Samples were kept at higher temperature for one hour showed a measurable decreasing on color intensity. It also detected a decreasing color intensity on sample heated at higher temperature (more than 100°C) for one hour. Moreover, the highest color intensity was from sample stored at 100°C for one hour while the lowest color intensity was from sample stored at 200°C for one hour. A similar effect of heating temperature was happened for all yellow, orange and red pigment.



**Figure 3. Effect of Heating Temperature to Color Intensity of Cassava-based Angkak Pigment**

## Effect of Ph

Another factor affected color intensity of angkak pigment is pH. Following results showed pH effect to color intensity of cassava-based angkak pigment in different pH solution in Table 4, 5 and 6. Observations were conducted at pH 4, 7 and 9 for 0, 3, 6, 9 and 12 hours.

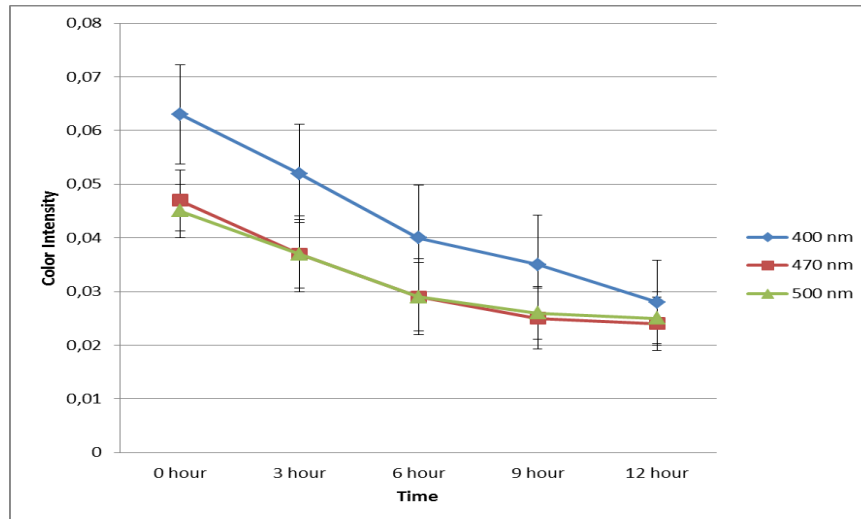


Figure 4. Color Intensity of Cassava-based Angkak Pigment in pH 4 for several hours

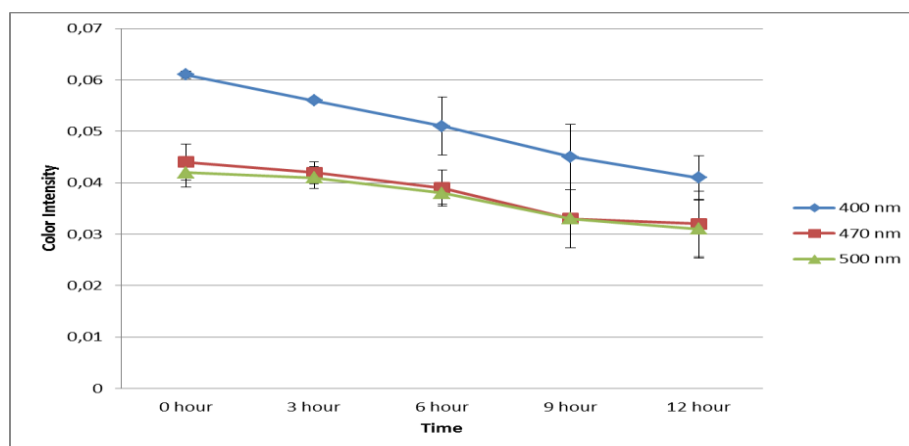


Figure 5. Color Intensity of Cassava-based Angkak Pigment in pH 7 for several hours

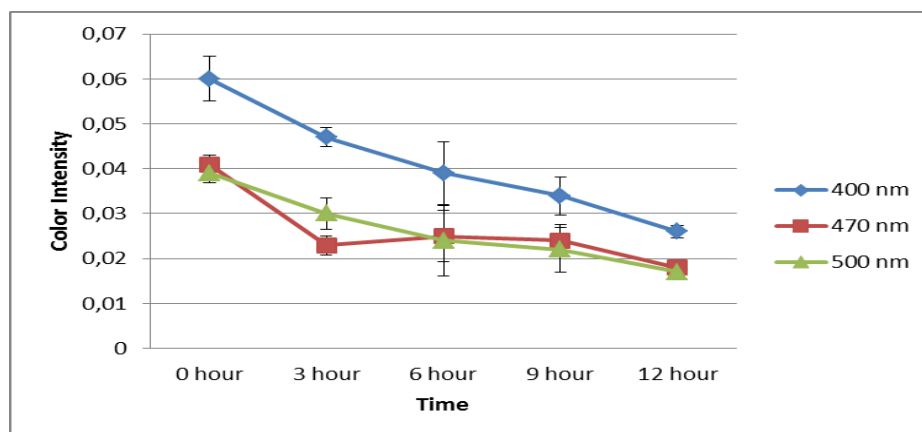


Figure 6. Color Intensity of Cassava-based Angkak Pigment in pH 9 for several hours

It can be observed from Fig.4, 5, and 6 that similar color intensity of angkak in pH 4 and pH 9 to pH 7 at initial condition (0 hour). However, decreasing of color intensity was significantly detected after a longer period with 3 hours interval. On the other hand, pigment stability lasted longer in pH 7 since a slightly decreasing of color intensity. Up to 12 hours, there was 2/3 of color intensity remained in pH 7 while in pH 4 and 9 were 1/3 only. Similar results were observed on 400 nm (yellow), 470 nm (orange) and 500 nm (red). It can be inferred that color intensity of angkak pigment lasted longer in pH 7 compared to pH 4 and pH 9 where color intensity decrease quicker during longer period in those pH solution.

### CONCLUSION

Pigment stability analysis on angkak pigment from cassava showed that increasing on angkak solubility along with increasing temperature up to 100°C. However, heating angkak pigment in boiled water for one hour or above 100°C can reduce color intensity of product. In similarly, color intensity was also decreasing at pH 4 and pH 9.

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