Effect of Molasses Concentration on the Pigment Production of *Monascus purpureus* M9 on Monascus Fermented Durian Seed

Andreas Alvin[1*], *Ira* Nugerahani[1] , *Susana* Ristiarini[1], *Indah* Kuswardani[1], *Ignatius* Srianta[1], and *Ihab* Tewfik[2]

[1]Department of Food Technology, Faculty of Agricultural Technology, Widya Mandala Catholic University Surabaya, Jalan Dinoyo 42-44 Surabaya, 60265, Indonesia
[2]School of Life Sciences, University of Westminster, 115 New Cavendish Street, London, W1W 6UW, UK

Abstract. Efforts have been made to cultivate *Monascus purpureus* on durian seed to produce Monascus Fermented Durian Seed (MFDS). MFDS pigment production could be enhanched by substrate addition by adding carbon source such as molasses. The purpose of this study was to determine the effect of molasses concentration on the production of *M.purpureus* M9 pigment in MFDS. The research used randomized block design with one factor, namely the concentration of molasses (M) with six treatment levels, M0 = 0%; M1 = 2%; M2 = 4%; M3= 6%; M4 = 8% and M5 = 10% with four repetitions. Findings showed that molasses addition had a significant effect on the color of MFDS powder, water soluble pigment level, 99.9% ethanol soluble pigment level and the pigment profile for both solvents. The best treatment based on pigment content was the M4 treatment with an 'L value' of 43.83, a* value of 14.92, b* value of 7.08, C value of 16.54, ^{o}H value of 24.45 and aquades soluble pigment content of 16.86AU/g (yellow); 9.23AU/g (orange); and 8.19AU/g (red) and ethanol soluble pigment content of 9.8AU/g (yellow); 2.5AU/g (orange); and 3.0 AU/g (red). Molasses is able to enhance pigment production of *Monascus purpureus* M9 of MFDS.
Keywords: Monascus Fermented Durian Seed, Molasses, Color, Pigment Profile

1. Introduction

In general, Angkak fermentation uses solid media such as rice, but *Monascus purpureus* can also grow on durian seed media [1]. Based on the results of research conducted by Srianta et al [2] on durian seeds with the TLC method, it produces a pigment profile which is divided into several colors including red, orange and yellow. The process of making Angkak goes through several stages such as soaking rice, packaging and closing in a glass container, sterilization, inoculation, fermentation, harvesting and drying [3].

*Corresponding author: Alvinwibisono@yahoo.co.id

The results of the research of Nimnoi et al. [4], showed that the production of *Monascus purpureus* pigment can still be increased by enriching nutrients in the form of C and N sources in the growth media. One of the substrates that can be used is molasses. Molasses can act as a source of C because it has a fairly high sugar content of 50% which consists of sucrose, glucose and fructose which are useful for growth, reproduction and production of pigment by *Monascus purpureus* [5]. Therefore, this study aimed to determine the effect of molasses concentration on the pigment production of *Monascus* fermented durian seed

2. Materials and Methods

2.1. Materials

The materials used include durian seeds of petruk variety obtained from pastry sellers in Surabaya, $Ca(OH)_2$ (technical grade), pure culture of *Monascus purpureus* M9, Potato Dextrose Agar (Merck 1.10130.0500), Potato Dextrose Broth, molasses, peptone from meat (Merck), distilled water, chloroform (Honeywell), methanol (Merck), and ethanol 99.9% (Merck)

2.2. Methods

Durian seeds are first washed and sorted by size. Then weighed and boiled in 5% $Ca(OH)_2$ solution. After that, it was washed, peeled and cut into cubes (±1cm3). After being cut, the durian seeds were put into an erlenmeyer flask as much as @50g and the molasses concentration was added according to the treatment. This mixture was then sterilized at 121^{o}C, 15 lbs/inch^2 for 15 minutes. Sterile media was inoculated with 5% starter of *M. purpureus* M9 and fermented at 30^{o}C for 14 days. After 14 days, the durian seeds were harvested and dried at 45^{o}C for 24 hours then crushed and sieved through a 40 mesh sieve.

2.2.1. Color Analysis of Monascus Fermented Durian Seed

The sifted durian seed extract was placed in a plastic bag and then the L, a*, b*, C and ^{O}H values were read using the Konika Minolta CR-20 color reader.

2.2.2. Pigment Level Analysis with Aquadest and 99,9% Ethanol solvents

Monascus fermented durian seed (MFDS) was extracted using aquadest and 99.9% ethanol as a solvent with a ratio of Angkak powder: solvent 1:15 for aquadest solvent and 1:5. For 99% ethanol solvent. Extraction was carried out by shaking water bath with rotation of 100 rpm for 2 hours at 30^{o}C, then filtered with Whatmann filter paper no.40. The filtrate obtained was then diluted 5 times and the absorbance was measured using a spectrophotometer with λ= 400 nm (yellow), λ= 470 nm (orange) and λ= 500 nm (red). Absorbance data is expressed in Absorbance Units per gram of sample (AU/g).

2.2.3. Pigment Profile Analysis

Pigment profile analysis was carried out by spotting 60 drops of water-soluble durian seed filtrate and 30 drops for 99.9% ethanol solvent on the stationary phase of 60F254 silica gel plate. The spotted silica plate is inserted into the chamber which contains the mobile phase in the form of a mixture of chloroform: methanol: water (90: 25: 4). When the mobile

phase reaches the specified limit, the silica plate is removed. Furthermore, observations and calculations of the retention factor (RF) were carried out.

3. Results and Discussion

3.1. Color Analysis of Monascus Fermented Durian Seed

Color analysis of MFDS powder was carried out using a color reader (Konika Minolta Cr-20) to determine the values of L (lightness), a (Redness), b (Yellowness), C (Chroma) and H (Hue). The results of color analysis of durian seed extract with a color reader can be seen in Table 1.1.

Table 1. Result of Color Analysis of Monascus Fermented Durian Seed powder with Color Reader

Treatment	L	a*	b*	C	°H
M0	50,57^{e}	13,53^{b}	3,53^{a}	14,00^{a}	14,47^{a}
M1	49,30^{e}	13,91^{c}	5,31^{b}	14,89^{b}	20,86^{b}
M2	47,15^{d}	14,23^{d}	6,15^{c}	15,53^{c}	23,29^{c}
M3	45,63^{c}	14,53^{e}	6,60^{cd}	15,95^{d}	24,50^{cd}
M4	43,83^{b}	14,92^{f}	7,08^{d}	16,54^{e}	25,45^{d}
M5	40,74^{a}	12,37^{a}	6,42^{c}	13,93^{a}	27,45^{e}

The results of Color Analysis of MFDS Powder with Color Reader showed that the higher the concentration of molasses, the lower the lightness (L) value. This was caused by an increase in the concentration of molasses added because the molasses has a dark brown color and can reduce the brightness level (L) of the durian seed. The a (redness) and b (yellowness) values showed an increase in the intensity of the red color from treatment M0 to M4 but decreased in treatment M5. this proves that the added molasses can stimulate the production of M. purpureus M9 pigment but in the M5 treatment there is a decrease which can occur due to the low L value, causing the perception of red and yellow colors to be low. The value of C also increased from treatment M0 to M4 and then decreased at the concentration of M5. This is in line with the values of a* and b* so that it can be seen that the M4 treatment has the highest level of red and yellow color saturation and is significantly different from other treatments. The value of C can be calculated using the formula $C = \sqrt{a^2 + b^2}$ so that the value of C will be proportional to the values of a* and b* [6]. The higher the concentration of addition of molasses, the higher the °H value detected, thus indicating an increase in the intensity of the reddish yellow color.

3.2. Pigment Level Analysis with Aquadest Solvent

Analysis of pigment level showed that the level of aquadest soluble pigments in treatment M0 to M4 increased, then decreased in treatment M5. The M4 treatment produced the highest level of water soluble pigments with absorbance of 16.86 AU/g (yellow); 9.23AU/g (orange); and 8.19AU/g (red) and significantly different from other treatments. The results of the analysis of water soluble pigment levels can be seen in Figure 1.1.

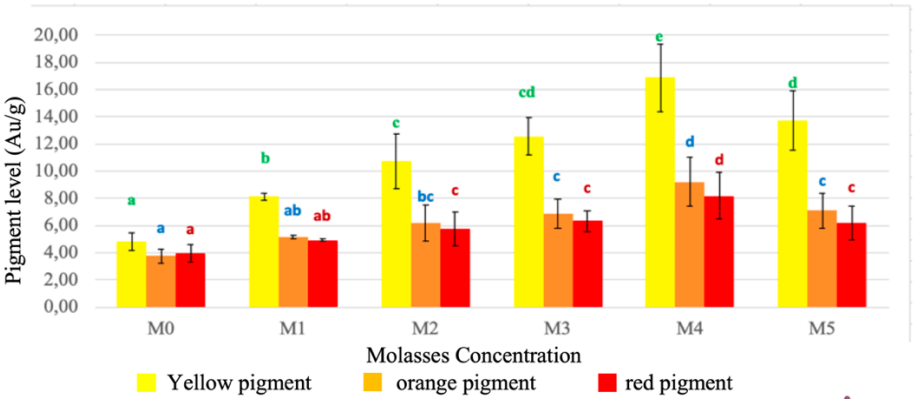

Fig.1. The results of the analysis of water soluble pigment levels (green font = yellow pigment notation; blue font = orange pigment notation; red font = red pigment notation)

This proves that the addition of molasses is able to increase the production of M. purpureus pigment in MFDS because the growth and pigment production of M. purpureus M9 is highly dependent on the composition of the carbon source present in the media [7]. However, the addition of molasses with a concentration that is too high can have an impact on decreasing the levels of pigment produced. This is because *M.purpureus* can only maintain a balance of osmotic pressure between 0.2 - 1Mpa and if the osmotic pressure exceeds this range, the turgor pressure in the cell can be disturbed, thereby disrupting the growth and production of pigment [9].

3.3. Pigment Level Analysis with 99,9% Ethanol Solvent

The levels of ethanol soluble pigments in the M0 to M4 treatments increased, then decreased in the M5 treatment (10% molasses). MFDS with M4 treatment produced the highest levels of ethanol soluble pigment with absorbance of 9.8 AU/g (yellow); 2.5 AU/g (orange); and 3.0 AU/g (red) and significantly different from other treatments. The results of the analysis of 99.9% ethanol soluble pigment levels can be seen in Figure 1.2.

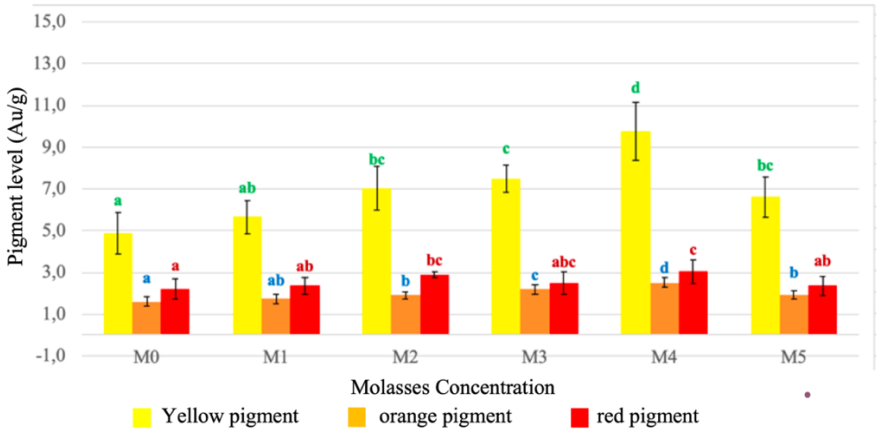

Fig. 2. The results of the analysis of 99,9% ethanol soluble pigment levels (green font = yellow pigment notation; blue font = orange pigment notation; red font = red pigment notation)

This proves that the addition of molasses is able to increase the production of M. purpureus pigment in MFDS. As previously explained, this can happen because M. purpureus is able to utilize the substrate as a source of C for its growth and pigment formation. In addition, the decrease in pigment content at a concentration of 10% is the result of higher osmotic pressure.

In this study, the overall levels of aquadest soluble pigments were higher than that of 99% ethanol soluble pigments. This can be caused by the concentration of ethanol that is too high. According to Srianta et al. [2], The optimum ethanol:aquadest ratio for the extraction of M. purpureus pigment is 7:3 (70% ethanol) and the unfavorable ethanol:water ratio used for *M. purpureus* pigment extraction is 10:1 (absolute ethanol). This can happen because different ratios of ethanol and aquadest have different levels of polarity.

The yellow pigment produced was higher than the other two pigments in both aquadest and ethanol solvents. This is thought to be caused by a low nitrogen source in the media and substrate, resulting in the formation of more yellow pigments compared to red pigments. The reason is based on the reaction of nitrogen bases with orange pigments that form red pigments, whereas molasses does not have a significant protein content to supply a source of N in the medium used. In addition, the orange pigment is also lower when compared to the yellow pigment. This can be caused because the yellow pigment is an alteration of the orange pigment.

3.4. Pigment Profile Analysis

In this study, the pigment profile was tested using the Thin Layer Chromatography (TLC) method with the stationary phase in the form of a silica gel plate (Merck) and the mobile phase which was a mixture of chloroform: methanol: aquadest (90: 25: 4). The results of the TLC test for the 99% ethanol soluble pigment profile and the TLC test results for the aquadest soluble pigment profile can be seen in Figure 1.3.

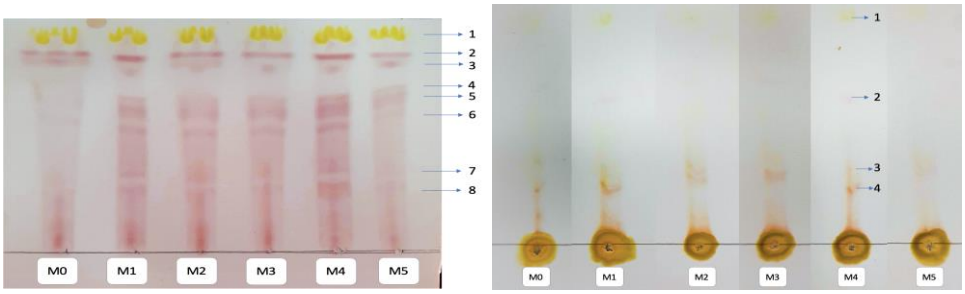

Fig. 3. TLC Test Results of 99% Ethanol Soluble Pigment Profile (Left) and TLC Test Results of Aquadest Pigment Profile (Right)

In testing the profile of the 99% ethanol soluble pigment, it is known that there is a separation between the red and yellow pigments with 8 spots and the retentin factor (Rf) value is between 0.31-1.00. Meanwhile, in testing the profile of aquadest soluble pigments, it is known that there is a separation between red and yellow pigments with 4 spots and an Rf value between 0.31-1.00. The color intensity of the highest pigment profile was found in the M4 treatment. The color intensity of the ethanol soluble pigment is 99.9% higher than that of the aquadest soluble pigment. This is due to a higher dilution factor for aquadest

solvent, namely 1:15 (MFDS) powder: distilled water) compared to 1:5 (MFDS powder: distilled water) due to the presence of natural gums found in durian seeds which can bind water and increase the viscosity of the solution [9]. This causes the durian seed extract to be difficult to filter if too little water is added. The types of pigments detected in ethanol solvent were more diverse (8 spots) compared to the pigments in aquadest solvent (4 spots) because *M. purpureus* pigments were slightly polar [8], so they were more soluble in ethanol solvents than in aquadest solvent.

4.1. Conclusion

The addition of molasses concentration significantly affected the color of the MFDS powder, the level of aquadest soluble pigment, the level of 99,9% ethanol soluble pigment, and aquadest and 99,9% ethanol soluble pigment profile. The most optimal treatment for the production of M. purpureus M9 pigment was M4 treatment with a value of L = 43.83; a* = 14.92; b* = 7.08; C = 16.54 and ºH = 25.45, along with aquadest-soluble pigment content of 16.86 AU/g (yellow); 9.23AU/g (orange); and 8.19AU/g (red), and 99.9% ethanol soluble pigment content of 9.8 AU/g (yellow); 2.5 AU/g (orange); and 3.0 AU/g (red). Pigment profile analysis showed that there were 8 spots of ethanol soluble pigment and 4 spots of water soluble pigment and the M4 treatment had the highest intensity of the pigment profile compared to other treatments.

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Reference

1. S.R.D. Puspitadewi, I. Srianta, N. Kusumawati, Pola Produksi Pigmen *Monascus* oleh *Monascus Sp*. Kjr 2 pada Media Biji Durian Varietas Petruk melalui Fermentasi Padat, *Jurnal Teknologi Pangan dan Gizi*. **15 (1),** 36-42 (2016)
2. Srianta, S. Ristiarini, I. Nugerahani, Pigments Extraction from Monascus-Fermented Durian Seed, *International Conference on Food and Bio-Industry*, 29-30 July 2019, Bandung, Indonesia (2019)
3. D.T. Tisnadjaja, *Bebas Kolesterol & Demam Berdarah dengan Angkak*. Jakarta: Penebar Swadaya, **22** (2006).
4. P. Nimnoi, N. Pongsilp, S. Lumyong, Utilization of Agro-Industrial Product for Increasing Red Pigment Production of Monascus Purpureus AHK12. *Chiang Mai J. Sci*. **42(2)**,331-338 (2015)
5. J.N. Runic, K.E.O. Connor, *Advances in Applied Microbiology*. London: Elsevier, **176** (2013)
6. M.R. McLellan, L.R. Lind, R.W. Kime, Hue Angle Determinations and Statistical Analysis for Multiquadrant Hunte L,a,b Data, *Journal of food quality*.**18**, 235-240 (1995)

7. P. Nimnoi, S. Lumyong, Improving Solid-State Fermentation of *Monascus purpureus* on Agricultural Products for Pigment Production, *Food Bioprocess Technol*. **4**, 1384-1390 (2011)
8. D.J. Davis, C. Burlak, N.P. Money, Osmotic Pressure of Fungal Compatible Osmolytes, *Mycol*. *Res*. **104 (7)**, 800–804 (2000)
9. Srianta, S. Ristiarini, I. Nugerahani, Pigments Extraction from Monascus-Fermented Durian Seed, *International Conference on Food and Bio-Industry*, 29-30 July 2019, Bandung, Indonesia (2019)
10. Srianta, Harijono, *Monascus*-fermented sorghum: pigments and monacolin K produced by *Monascus purpureus* on whole grain, dehulled grain and bran substrates, *International Food Research Journal*. **22(1),** 377-382 (2015)