

Yellow pigment fraction from Monascus-fermented durian seed: thermal stability and bioactivities

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Yellow pigment fraction from *Monascus*-fermented durian seed: thermal stability and bioactivities

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7

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Abstract

Angkak is a functional product made by fermenting rice with *Monascus purpureus*. Studies have shown that agro-industrial by-products such as durian seeds are able to support *Monascus* growth, thus the potential of producing *Monascus*-fermented products with similar functional activities as *angkak*. Utilization of durian seeds to produce *Monascus*-fermented durian seed (MFDS) would improve durian production sustainability. *Monascus* yellow pigment (MYP) fraction was separated from MFDS and evaluated for its thermal stability by time (30 and 60 mins) and temperature treatments (40°C, 60°C, 80°C and 100°C), antioxidant activity with 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity assay, and anti-diabetes activities by measuring α -glucosidase inhibition activity. MYP showed increasing stability when treated with increasing incubation temperature, ranging from 90.87 $\pm$ 0.35% to 96.33 $\pm$ 0.00% ($p < 0.05$). MYP reached the highest stability when incubated at 100°C for 60 mins. The antioxidant activity of MYP was detected to be 51.84 $\pm$ 0.17%. MYP showed anti-diabetes activity ranging from -0.72 $\pm$ 0.27% (10 μ g/mL) to 29.40 $\pm$ 4.27% (100 μ g/mL). There was an increase in α -glucosidase inhibition activity along with increasing MYP concentration. *Monascus* yellow pigments extracted from *Monascus*-fermented durian seed have the potential to be used as a functional ingredient in the food industry.

1. Introduction

Red yeast rice or commonly known as *angkak* in Indonesia, is a functional food product produced by fermenting rice with the yeast *Monascus purpureus*. *Angkak* has been proven to have a variety of functional activities, including anti-dyslipidemia, anti-diabetes, antihypertension, anti-obesity, and anti-inflammation (Nguyen *et al.*, 2017; Hu *et al.*, 2020). These activities are due to the bioactive compounds found in *angkak*, such as *Monascus* pigments, monacolins, unsaturated fatty acids, decalins, and aminobutyric acid (Zhu *et al.*, 2013). *Angkak* is widely used as food flavouring, colorant, and traditional medicine in Asian countries (Song *et al.*, 2019). Studies have shown that *Monascus* sp. is able to grow on other substrates besides rice, particularly agro-industrial by-products i.e. corn bran, potato peel, coffee residue, and durian seeds (Almeida *et al.*, 2021; Mousa *et al.*, 2018; Brito *et al.*, 2012; Srianta *et al.*, 2021).

Durian (*Durio zibethinus*) is a highly popular fruit that grows in tropical countries like Indonesia. According to the Indonesian Central Bureau of Statistics

(Badan Pusat Statistik, 2022), the production of durian in Indonesia has increased 53.06% during the last ten years, increasing from 0.8 million tons in 2011 to 1.3 million tons in 2021. Non-edible durian parts can be up to 70%, consisting of 50-60% durian shells and 10-20% seeds, which are discarded as waste and not widely utilized (Purnomo *et al.*, 2016). Srianta *et al.* (2021) found that the moisture content of durian seeds (around 60%) is highly suitable for *Monascus purpureus* growth. Durian seeds have good potential as an alternative substrate for *angkak* production. The utilization of durian seeds to produce functional food products such as *angkak* could help lessen industry waste and improve durian production sustainability.

Monascus-fermented durian seed (MFDS) is a product of durian seed fermented with *Monascus purpureus*. Previous researchers have shown that MFDS has antioxidant and anti-diabetes activities (Subianto *et al.*, 2014; Nugerahani *et al.*, 2017; Srianta *et al.*, 2021), which was highly suspected to come from the *Monascus* pigments produced, alongside phenols and monacolin K. The bioactivities that MFDS has shown raise the

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potential of MFDS pigments being used in the food industry as a functional and coloring additive. In its application for food products, thermal stability is an important characteristic of *Monascus* pigments to have. MFDS pigments, which have high solubility in ethanol, need further research on their stability to measure the pigments' capability on being applied to products that require thermal processing.

Out of the major pigments produced by *Monascus purpureus* (yellow, orange and red), yellow pigments were the most found in MFDS (Srianta, Ristiarini and Nugrahani, 2020). *Monascus* yellow pigments (MYP) in particular have also been widely researched in recent years due to their anti-cancer, anti-tumor, anti-inflammatory and anti-obesity effects (Chen and Wu, 2016). Some researchers reported that MYP compounds, i.e. monascin, ankaflavin and monaphilone A had shown antioxidant activity (Hsu et al., 2013; Shi et al., 2016; Wu et al., 2021). Shi et al. (2012) found that monascin is able to attenuate symptoms of diabetes and extend the lifespan of streptozotocin-induced diabetic rats by enhancing oxidative stress resistance. Ankaflavin has been reported to reduce advanced glycation end-products and promote insulin sensitivity and production in methylglyoxal-induced rats by inhibiting inflammation (Lee et al., 2012). Based on the previously mentioned studies, yellow pigments produced from *Monascus*-fermented durian seed have received interest as being the pigment contributing to MFDS bioactivities.

Srianta, Nugrahani and Ristiarini (2020) have separated the yellow pigment fraction from *Monascus*-fermented durian seed, however, its characteristics have not yet been known. Yellow pigments from MFDS have the potential to be a functional food ingredient with a sustainable substrate. This research aimed to evaluate the characteristics of the *Monascus* yellow pigment fractions produced on durian seed substrate i.e. thermal stability, antioxidant, and anti-diabetes activities.

2. Materials and methods

2.1 Materials

Monascus purpureus M9 culture was used in the solid-state fermentation on durian seed substrate. The culture was maintained periodically on Potato Dextrose Agar. Durian seeds (*Manalagi* variety) were obtained from a durian processing unit in Surabaya (Indonesia) and stored in a freezer (-4°C) before use. All chemicals used were chromatographic and analytical grades.

2.2 Preparation of *Monascus purpureus* fermentation product from durian seed

This research was carried out in 2020 at Widya

Mandala Surabaya Catholic University laboratories. The preparation of MFDS was based on previous research by Srianta et al. (2012). The durian seeds were washed, boiled in 5% (w/v) Ca(OH)₂ solution for 10 mins, peeled to remove the seed coat, and cut into 1 × 1 × 1 cm sized pieces. The durian seeds weighed 50 g for each 300 mL flask. The seeds were sterilized in an autoclave (121°C for 15 mins), cooled to room temperature, and inoculated with 5% (w/v) of starter culture. After 14 days of incubation at 30°C with manual daily shaking, the fermented matter was dried at 45°C for 24 hrs, ground, and then sieved.

2.3 Yellow pigment extraction and separation

Extraction and separation of MYP were carried out according to Srianta, Nugrahani and Ristiarini (2020). MYP was extracted with the soxhletation method in ethanol solvent at a ratio of 1:50 for 2 hrs. The extract was then evaporated in a rotary evaporator. The evaporated extract was subjected to a separation process. MYP separation was performed in column chromatography (10 mm × 500 mm) with silica gel 60. The column was packed with silica gel by slurry method, then pre-eluted with ethyl acetate-ethanol-water (90:25:4) solution. The extract was put into the column and eluted with the same ratio of ethyl acetate, ethanol and water. The MYP fraction was subjected to evaluation of thermal stability, antioxidant activity and anti-diabetes activity.

2.4 Experimental design

The 4 × 2 factorial design was used to determine the effect of a combination of two factors (incubation temperature and time) towards the thermal stability of MYP from MFDS. Incubation temperatures of 40°C, 60°C, 80°C, and 100°C for times of 30 and 60 mins were studied. Data of thermal stability was collected in four replications and triplicated for each replication.

2.5 Determination of thermal stability

Determination of pigment stability was carried out according to Carvalho et al. (2005). The alcoholic solution of MYP in stoppered glass tubes was incubated at different temperatures for different times. The color intensity was read as absorbance at 400 nm directly for each tube against ethanol as blank using a spectrophotometer. Thermal stability percentages were determined by dividing the obtained absorbance for each tube, all along incubation time, by the initial absorbance of the same tube and multiplying the results by 100%.

2.6 Determination of bioactivities

In vitro antioxidant activity was determined with 2,2

-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity assay according to Tseng *et al.* (2006). The sample extract (3.8 mL) was added to 0.2 mL DPPH (50 mg in 100 mL methanol) in a wrapped glass tube. The tube was left in a dark room for 30 mins. The absorbance was measured at 517 nm. The inhibition percentage (%) was calculated with the control basis of the DPPH solution's absorbance value.

In vitro anti-diabetes activity was determined with α -glucosidase inhibition activity according to Artanti *et al.* (2012). The sample (0.1 mL) was added to a test tube containing 0.1 mL of 20 mM pNPG (p-Nitrophenyl α -D-glucopyranoside) and 2.2 mL of 100 mM phosphate buffer at pH 7.0, then incubated for 5 mins at 37°C. The reaction was initiated by the addition of 0.1 mL of enzyme solution (1 mg/0.1 mL) followed by 15 mins incubation at 37°C. The reaction was stopped by the addition of 2.5 mL of 200 mM Na₂CO₃. The absorbance of p-nitrophenol released from PNPg at 400 nm was measured with a spectrophotometer. The percentage of inhibition on the α -glucosidase activity was calculated by the equation:

$$\% \text{ Inhibition} = [1 - (B/A)] \times 100\%$$

Where A is the absorbance in the absence of the sample and B is the absorbance in the presence of the sample.

2.7 Data analysis

The obtained data was calculated for the average and standard deviation. The statistical significance of the thermal stability data was determined by two-way Analysis of Variance (ANOVA) at $\alpha = 5\%$ with IBM SPSS Statistics (version 19.0). Further analysis with Duncan's Multiple Range Test (DMRT) at $\alpha = 5\%$ was performed when the data were significantly different.

3. Results and discussion

3.1 Thermal stability

MYP has been extracted from MFDS and incubated at different temperatures for different times to analyze its thermal stability. The stability of the pigment at different temperatures and times is shown in Table 1. MYP from MFDS had high thermal stability, as it managed to remain stable as high as 96.33% during thermal treatments. Carvalho *et al.* (2005) found that *Monascus* pigments in ethanol had no significant changes in color after incubating at temperatures as high as 100°C. Similar results were found in *Monascus* pigments produced from *Monascus* fermentation of alternative substrates besides rice. Suraiya *et al.* (2017) reported that *Monascus* pigments from *Saccharina japonica*

fermented by *Monascus purpureus* were thermostable at 10-100°C, retaining over 85% of pigments after 1 hr of incubation. Pigments from *Monascus* fermented corn cob were also stable after autoclaving and exposure to dry heat (Velmurugan *et al.*, 2011). MYP had the lowest thermal stability of 90.87% when incubated at 40°C for 60 mins, while incubation at 100°C for 60 mins resulted in the highest thermal stability percentage (96.33%). Studies have shown increasing temperature would increase *Monascus* pigment degradation (Carvalho *et al.*, 2005; Nimnoi and Lumyong, 2011; Vendruscolo *et al.*, 2013; Abdollahi *et al.*, 2021). However, statistical analysis showed a pattern where MYP that had been incubated in higher temperatures (80 and 100°C) were more stable for longer periods of time (60 mins) in comparison to MYP incubated at lower temperatures (40 and 60°C). All around, MYP extracted from MFDS showed higher stability at higher temperatures. Durian seed as a substrate is capable of producing highly thermostable yellow pigments from *Monascus* fermentation, which has the potential to be used on food products processed at high temperatures such as bakery products and beverages (Subianto *et al.*, 2014).

Table 1. Thermal stability of *Monascus* yellow pigment (%).

Temperature (°C)	Incubation time	
	30 mins	60 mins
40	93.66±0.00 ^b	90.87±0.35 ^a
60	94.66±0.00 ^d	94.16±0.00 ^c
80	95.27±0.19 ^e	95.83±0.16 ^f
100	95.94±0.19 ^f	96.33±0.00 ^g

Values are presented as mean±SD. Values with different superscripts are statistically significantly different (p<0.05).

3.2 Bioactivities

Recent interest in the use of *Monascus* pigments, yellow pigments in particular, is because of their bioactivities for human health, which include antioxidant and anti-diabetes activities. The antioxidant activity of MYP from MFDS was determined by its DPPH inhibition activity, whereas α -glucosidase inhibition activity was used to determine anti-diabetes activity (Kim *et al.*, 2007; Subianto *et al.*, 2014). The results are shown in Table 2. MYP was found to have antioxidant activity by exhibiting 51.84% of DPPH inhibition activity. Other studies have found similar results. Yellow pigments from *Monascus ruber* and *M. purpureus* had shown DPPH scavenging activity with lower, but comparable results to butylated hydroxytoluene (BHT) as the positive control (Tan *et al.*, 2018; Amany and Abdel-Raheem, 2020). Certain yellow pigments might have contributed to MYP of MFDS' antioxidant activity as a whole. Monascin, ankaflavin, monaphilone A, monaphilone B, and monasfluore B were found to have

antioxidant activity through *in vitro* and cell model tests. An increase in the antioxidant capacity of MYP would happen along with an increase in concentration (Zhang *et al.*, 2020; Wu *et al.*, 2021). Both monascin and ankaflavin were detected in the MFDS extract (Srianta, Ristiarini and Nugrahani, 2020). Wu *et al.* (2020) reported that C-H bonds located in two out of the three rings of monascin were likely to be involved in the antioxidant process.

Table 2. DPPH and α -glucosidase inhibition activity.

Bioactivity	Value (% inhibition)
DPPH inhibition activity	51.84 $\pm$ 0.17
α -Glucosidase inhibition activity	
MYP fraction concentration (μ g/mL):	
10	-0.72 $\pm$ 0.27
25	3.58 $\pm$ 0.37
50	14.96 $\pm$ 3.39
100	29.40 $\pm$ 4.27
Quercetin concentration (μ g/mL):	
1	33.31 $\pm$ 0.70
2.5	55.39 $\pm$ 1.03
5	68.80 $\pm$ 1.02
10	80.43 $\pm$ 0.48

Values are presented as mean $\pm$ SD.

MYP showed α -glucosidase inhibition activity at the concentration of 25 μ g/mL (3.58%). As the MYP concentration increases, the inhibition percentage experiences an upward trend as well. The highest α -glucosidase inhibition percentage (29.40%) was exhibited by 100 μ g/mL of MYP. Inhibiting α -glucosidase, an enzyme that catalyzes carbohydrate hydrolysis, would prevent glucose production, thus indicating that the MYP from MFDS has anti-diabetes activity. Similar results have been reported by Kim *et al.* (2007), who found that red *Monascus* pigment derivatives had shown inhibitory activities against α -glucosidase. The pigments' ability to inhibit α -glucosidase was assumed to be due to the binding of the pigments to α -glucosidase by hydrogen bonding interaction between hydroxyl groups and amino acids. Monascin and ankaflavin especially could have contributed to MYP's α -glucosidase inhibition activity as they have been proven to exhibit anti-diabetes activity (Shi *et al.*, 2012; Lee *et al.*, 2012; Chen and Wu, 2016).

In order to further explore the effectiveness of MYP's anti-diabetes activity, a comparison to quercetin's α -glucosidase inhibition activity has been made. Quercetin is a flavonoid widely known to have high α -glucosidase inhibition activity (Proenca *et al.*, 2017). In this study, it was able to reach up to 80.43% inhibition with increasing concentration. More concentration is needed of MYP to be able to inhibit α -glucosidase in comparison to quercetin. In fact, 10 μ g/

mL of MYP did not exhibit any inhibition activity whereas even 1 μ g/mL of quercetin was able to show more inhibition activity than 100 μ g/mL of MYP (33.31% and 29.40%, respectively). Quercetin contains hydroxyl groups in its molecular structure, which are able to act as hydrogen bond donors and enhance inhibitory activity (Lu *et al.*, 2020). A study has shown that flavonoids without any hydroxyl groups in their structure had no α -glucosidase inhibition activity (Proenca *et al.*, 2017). However, MYP's α -glucosidase activity cannot be ignored. MYP will be able to show higher inhibition activity (over 50%) when its concentration is increased to more than 100 μ g/mL.

4. Conclusion

Monascus yellow pigments extracted from *Monascus* -fermented durian seed showed the highest thermal stability of 96.33% when incubated at the highest temperature of 100°C for 60 mins. MYP exhibited 51.84% of DPPH inhibition activity. α -glucosidase inhibition activity increased with increasing MYP concentration, from 3.58% of 25 μ g/mL to 29.40% of 100 μ g/mL MYP. The results show the potential of MFDS yellow pigments to be used as a functional ingredient in food products such as bakery products and beverages.

Conflict of interest

The authors declare no conflict of interest.

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