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Research article

Effect of banana peels and phenolic compounds on pigments and citrinin production by *Monascus purpureus*

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Abstract

Importance of the work: Each phenolic compound exhibits different effects on pigments and citrinin production.

Objectives: To evaluate the effect of phenolic compounds in Hom Thong banana peel on pigments and citrinin production by *Monascus purpureus*.

Materials & Methods: The effects were evaluated of Hom Thong banana peel and its phenolic compounds on fungal growth, pigment and lovastatin production and mycotoxin citrinin reduction by *M. purpureus* TISTR 3003. The total phenolics and flavonoids contents were determined at two stages of harvest (mature green and overripe).

Results: Mature green Hom Thong banana peel contained the highest contents of total phenolics and flavonoids. The major phenolic compounds from banana peel were identified using high-performance liquid chromatography. Five phenolic compounds were found: gallic acid, caffeic acid, coumaric acid, catechin and rutin. Coumaric acid was found only in overripe banana peel and had an inhibitory effect on pigment production by *M. purpureus*. The highest lovastatin content (861.91 µg/g of dry weight) was produced from the optimized cultivation conditions of *M. purpureus* on overripe banana peel at 25°C for 32 d. The production of pigments and lovastatin was related to various factors, such as the ripening stage of the substrate and the incubation time.

Main finding: Phenolics within Hom Thong banana peel can reduce citrinin production. Furthermore, banana peel can be used as a substrate for lovastatin and pigment production under solid state fermentation by *M. purpureus* as a safe, active ingredient in food products.

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Introduction

Monascus sp. is a fungus that produces color in foods such as red yeast rice, fermented bean curd, and sausage and it is also used as traditional medicine, especially in the Asian region (Ma et al., 2000). Red yeast rice produced from *Monascus* sp. is a dietary supplement product that originated in China more than 1,000 years ago and is widely known around the world. Natural food colorants are produced by *Monascus* sp. and they are considered as secondary metabolites that are not related to the important role of metabolism of living organisms (Ma et al., 2000; Lee et al., 2006). Lovastatin is produced by *Monascus* sp. during the stationary phase (Patakova, 2013). Pigments produced from *Monascus* sp. can be used as food coloring instead of artificial colors, reassuring health-conscious consumers regarding the safety of food products they eat. However, there have been reports (Liao et al., 2014) of contamination by citrinin toxin in food supplements and products from *Monascus* sp., which had higher values than the Food and Drug Administration Taiwan upper limits for citrinin in red yeast rice products (5,000 µg/kg) and in *Monascus* products (2,000 µg/kg).

Citrinin is produced from *Monascus* sp. and can have an impact on the liver and kidneys of humans (Orozco and Kilikian, 2008). Health problems may arise when food or agricultural raw materials that have been contaminated with citrinin are consumed over a long period of time; thus, there have been studies to establish a methodology to inhibit or destroy citrinin found in several products, so that those products are safe for consumption. For example, research on the reduction of citrinin production by *Monascus* sp. has involved study on controlling the conditions and pH level of the production process (Orozco and Kilikian, 2008). Vázquez et al. (2001) tested eugenol, a phenolic compound and a main component in cloves, for its effectiveness in controlling the production of citrinin. They found that eugenol at a concentration of 100 µg/mL could reduce the production of citrinin by *Monascus* sp. and eugenol at a concentration of 200 µg/mL inhibited the normal growth of *Penicillium citrinum*, with a long lag phase and it only started to grow after the ninth day. This was possibly caused by the hydrogen bonding formed between the hydroxy group of eugenol and the thiol group of fungal enzymes, resulting in deactivation of the enzyme and the death of the fungus (Park et al., 2007). In addition, Mossini and Kemmelmeier (2008) tested the inhibition of growth of *P. citrinum* mycelia by growing fungi on potato dextrose agar (PDA) mixed with 12.50 µg/mL of neem leaf extract, compared to the control group without the neem leaf extract. It was found that the colony size in the PDA with the neem leaf extract had a greater diameter than that of the control group. The main phenolic compounds found in neem leaves were gallic acid and ferulic acid (Singh et al., 2005).

Banana peel has a total phenolic content in the range 4.95–47.00 mg gallic acid equivalent (GAE)/g dry matter (González-Montelongo et al., 2010; Hernández-Carranza et al., 2016; Vu et al., 2018) depending upon many factors, such as the variety of banana, the cultivation area, the ripening phase and the extract solution. The ripening phase affects the phenolic content when the banana starts to ripen and the phenolic content decreases (Sundaram et al., 2011; Vu et al., 2018). There are more than 40 types of phenolic compounds in bananas. Flavan-3-ols, such as catechin, are the largest group of phenolic compounds found in banana peels (Rebello et al., 2014; Vu et al., 2018). Each type of phenolic compound has different effects on cells. For example, carvacrol disturbs ion transport via a protein that helps transport ions embedded in the cell membrane, which functions in passing through ions such as H^+ and K^+. When ions cannot pass through membranes, cell stress results, leading to cellular abnormality. Eugenol disturbs the movement of compounds through cell membranes resulting in cell stress conditions (Ansari et al., 2013).

The current research studied the types of phenolic compounds found in mature green and overripe Hom Thong banana peel and the effects of Hom Thong banana peel and phenolic compounds on the growth, pigment production and the production of citrinin toxin by *M. purpureus* in order to evaluate the potential for using Hom Thong banana peel to reduce toxin production by *M. purpureus* and to reuse leftover banana peels to obtain maximum benefit.

Materials and Methods

Raw materials and microorganisms

Hom Thong banana peels were obtained from King Fruits Co., Ltd. (Bangkok, Thailand) in February 2018. Both mature green and overripe banana peels were used. *M. purpureus*, variety TISTR 3003, was obtained from the Thailand Institute of Scientific and Technological Research.

Chemical composition of Hom Thong banana peel

Composition analysis of mature green and overripe Hom Thong banana peel samples was carried out to determine the concentration of nitrogen free extract and the crude protein, lipid, crude fiber, ash and moisture contents, according to the methods of Association of Official Analytical Chemists (2000). All measurements were done in triplicate. The concentration percentages were calculated.

Extraction and volumetric analysis of phenolic compounds

The phenolic compounds were extracted from mature green and overripe Hom Thong banana peels after sterilization. The banana peels used in the study were cleaned and disinfected in an autoclave at 121°C for 15 min. Afterward, the banana peels were dried at 50°C for 18 hr. Banana peel samples (1 g each) were extracted using 70% ethanol (10 mL) in an ultrasonic bath at a frequency of 37 KHz at 40°C. After 15 min, the extract was passed through 0.45 µm filter paper and the filtrate was analyzed for phenolic compounds. Each extraction process was done at least in triplicate. Each experimental result was expressed as the mean ± SD.

Quantity analysis of the total phenolic content was adapted from the Folin-Ciocalteu colorimetric method. According to the method of Wolfe et al. (2003); the calculated values were compared with the standard curve of gallic acid, presenting results in micrograms of gallic acid equivalents (GAE) per gram of dry sample.

Quantity analysis of the total flavonoids was performed according to the method of Wolfe et al. (2003); values were compared with the standard curve of catechin, being calculated and presented in micrograms per catechin equivalents (CE) per gram of dry sample.

Quantity analysis of the phenolic compounds was done using high-performance liquid chromatography (HPLC) under the following conditions: 1) Symmetry C_{18}, 4.6 × 250 mm, particle size 5µm; and 2) ultraviolet (UV) detector with wavelength of 280 nm, gradient program (0–20 min 10–60% B, 20–50 min 60–90% B) with 0.1% formic acid in water, where solvent A was acetonitrile and the flow rate was 1 mL/min, according to the method of Aboul-Enein et al. (2016). All standards of phenolic acids (gallic acid, caffeic acid, coumaric acid) and flavonoids (catechin, rutin) were obtained from Sigma-Aldrich (St. Louis, USA).

Study of effects of phenolic compounds on growth, pigments and citrinin productions

Cultivation of the fungus in broth medium was conducted using spore suspension of 1 mL at a concentration of 1×10^6 spores/mL placed in 25 mL of potato dextrose broth (PDB) in a 125 mL Erlenmeyer flask, which had been disinfected in an autoclave at 121°C for 15 min. Five different standard phenolic compounds that were found in the Hom Thong banana peels (gallic acid, caffeic acid, coumaric acid, catechin, rutin) were separately added at 1 mg/mL. Hom Thong banana peel powders (mature green, overripe) were separately added at 75 mg/mL. The fungus was cultivated at 25°C for 10 d. According to the method of Zhang et al. (2015), the PDB culture medium was filtered using Whatman filter paper no. 1 after cultivation to separate the mycelia. Later, the medium samples were dried and weighed. The weight of each sample was compared to that of the control group for pigment intensity and quantity analysis of citrinin toxin.

The pigment intensity was measured using mycelia that had been cultured in PDB medium for 10 d and recovered by filtering through Whatman filter paper no.1. The cultured medium was measured for its pigment intensity at wavelengths of 500 nm and 400 nm using a UV-vis recording spectrophotometer (A_{OD}). The pigment intensity (measured in units per gram dry weight, DW) was calculated using Equation 1:

$$\text{Pigment intensity} = A_{OD} \times \text{Dilution} \times \text{Volume of extract} \quad (1)$$

Extraction of citrinin was performed according to the method of Vázquez et al. (2001). The cultured PDB medium from the fungal culture (5 mL) was added to chloroform (15 mL), shaken and then left for separation. The chloroform layer was collected and evaporated to dryness. Afterward, the solution was prepared by dissolving the extract in methanol to analyze the amount of citrinin according to the method of Pattanagul et al. (2008) using HPLC. The conditions of analysis were: 1) Supelco (St. Louis, USA) C_{18} packed column 4.6×250 mm 5µm; 2) fluorescence detection at Xλ 331 nm eλ 500 nm; and 3) mobile phase consisting of distilled water at pH 2.5 (adjusted with phosphoric acid, 50%) and acetonitrile (50%) at a flow rate of 1 mL/min. Each experimental result was expressed as the mean ± SD. Statistical analysis of differences was performed using Duncan's test in the SPSS statistical software package.

Study of factors in cultivation of M. purpureus with banana peel

For the cultivation of *M. purpureus* using banana peel as substrate, a factorial experiment was performed in a completely randomized design with two factors: the ripening stage of Hom Thong bananas (mature green, overripe) and the incubation time (24 d, 28 d, 32 d, 36 d). The banana peels (20 g each sample) were dried and packed into polypropylene bags and disinfected in the autoclave. Afterward, a starter culture was prepared using a spore suspension of 5 mL at a concentration of 1×10^6 spores /mL, adjusted to an initial moisture content of 55% (weight per weight).

Measurement of pigment intensity was carried out using the method of Huang et al. *(2017). Briefly, fermented banana peels (1 g) were extracted with 70% ethanol (10 mL) using a shaker at a speed of 200 revolutions per minute for 30 min at 60*℃. *Afterward, the solution was passed through Whatman filter paper no.1 and pigment analysis* was conducted. The pigment intensity was analyzed at wavelengths of 500 nm and 440 nm using Equation 1 above.

Extraction of lovastatin and citrinin was performed by extracting dried fermented banana (1 g) with 70% ethanol (20 mL) in an Erlenmeyer flask. The supernatant was collected. A sample (1 mL) of the extract was filtered through a 0.45 µm cellulose acetate membrane and then used in determination of lovastatin and citrinin based on HPLC. Lovastatin (mevinolin) and citrinin were obtained from Sigma-Aldrich (St. Louis, USA) and dissolved in methanol to prepare the standard curve. Citrinin analysis was carried out using the method of Pattanagul et al. (2008). Quantity analysis of lovastatin was performed according to the method of Panda et al. (2010) using HPLC with the following conditions of analysis: 1) Symmetry Shield (Milford, USA) $C_{18,}$ 4.6×250 mm, particle size 5 µm; 2) UV detector at 235 nm; 3) mobile phase consisting of distilled water at pH 2.5 (adjusted with phosphoric acid, 30%) and acetonitrile (70%) at a flow rate of 1 mL/min.

All cultivations were carried out in triplicate and the results were presented as the averages of these values.

Statistical analysis

Analysis of variance was employed for data analysis of each experiment. Then mean values were compared based on Duncan's multiple range test and differences were considered significant at $p < 0.05$.

Results and Discussion

Chemical compositions of Hom Thong banana peels

From the analysis of the chemical composition of mature green banana peels and overripe banana peels using proximate analysis (Association of Official Analytical Chemists, 2000), the carbohydrate, crude protein, lipid, crude fiber, ash and moisture contents were determined to evaluate the suitability of the banana type as a source of growth and production of bioactive substances of *M. purpureus*. It was found that the mature green banana peels had carbohydrate, crude protein, lipid, crude fiber, ash and moisture contents of 46.01%, 8.45%, 3.71%, 19.45%, 17.51% and 4.87%, respectively, while for the overripe banana peels, they were 47.45%, 7.97%, 4.12%, 15.49%, 15.75% and 9.22%, respectively (Table 1). These amounts were consistent with Abubakar et al. (2016) who studied the chemical composition of overripe banana peels of a Nigerian variety, where the banana peels had mean ± SD values of 32.39 ± 0.70% carbohydrate and 5.53 ± 0.11% protein, but the lipid concentration was 23.93 ± 0.68%, which was higher than that for Hom Thong banana peels. The difference in lipid concentration may have been due to the different variety of banana used.

The chemical composition of cultivated Namwa banana grown in Thailand was more similar to the current results than for a variety from overseas based on the study by Rugthaworn et al. (2017). The overripe banana peels of Namwa banana contained protein of 6.23 ± 0.43% and lipid of 5.93 ± 0.03%, similar to Hom Thong banana peel. However,

Table 1 Proximate analysis of nutritional contents in Hom Thong banana peel agro-waste

Banana peel	Concentration (%)					
	NFE	Crude protein	Lipid	Crude fiber	Ash	Moisture content
Mature green	46.01	8.45	3.71	19.45	17.51	4.87
Overripe	47.45	7.97	4.12	15.49	15.75	9.22

NFE = nitrogen free extract

the Namwa banana peels had a higher amount of carbohydrates (57.65 ± 0.29%). Polysaccharide is a source of carbon suitable for growth of yeast and mold and for the fermentation of *Monascus* sp. for production of pigment and lovastatin (Rugthaworn et al., 2017).

Content of phenolic compound in banana peel

The banana peels in the current study contained high amounts of phenolic compounds, especially the mature green banana peels that had a total phenolic content of 59.74 mg GAE/g crude extract and a flavonoid content of 27.59 mg CE/g crude extract (Table 2). These amounts were consistent with another study of mature green banana peel (*Musa acuminata* L.) by Fatemeh et al. (2012) in Indonesia, where extracts from mature green banana peel had total phenolic contents higher than that of overripe banana peel (685.57 GAE/g crude extract and 586.29 mg GAE/g crude extract, respectively). These values of total phenolic contents were higher than those in the Hom Thong banana peel in the current study, perhaps due to the differences in the extraction techniques, variety and geographical cultivation. The current results were similar to other studies in Thailand, such as for Hom Thong banana cultivated in Phetchaburi province, where mature green banana peel had a total phenolic content of 23.71 mg GAE/g crude extract, which was also higher than that for overripe banana peel (Charoenteeraboon et al., 2019).

Types of phenolic compounds in Hom Thong banana peel

It was expected that phenolic compounds should have an effect on the growth of the fungus because several studies have reported the potency of phenolic compounds to inhibit the growth of several microorganisms (Bokhari, 2007). The mature green and overripe Hom Thong banana peels in the current study contained phenolic acids (gallic acid, caffeic acid, coumaric acid) and flavonoids (catechin, rutin), as shown in Fig. 1. The mature green banana peel had higher contents of gallic acid and catechin than the overripe banana peel (Table 3), whereas the overripe banana peel had a higher content of rutin than the mature green banana peel. The most obvious difference in content was for coumaric acid, which could only be found in the overripe banana peel. Both the mature green and overripe banana peels had high contents of catechin and rutin, which was consistent with other research studies on banana peel. For example, flavan-3-ol is a common flavonoid found in banana peel. Pramote et al. (2018) investigated the phenolic compound in Namwa banana peel (*Musa sapientum* L.) using HPLC and identified gallocatechin, catechin, epicatechin, and epigallocatechin as a series of flavan-3-ols. In the current experiment, mature green and overripe banana peels contained catechin contents of 39.76 ± 2.63 mg/g DW and 24.29 ± 1.82 mg/g DW, respectively. This was consistent with the finding that the total phenolic and flavonoid contents were higher in mature green banana peel than overripe banana peel. The levels in the current study were consistent with the phenolic compounds in banana peel samples of nine varieties (*Musa* sp.) investigated by Tsamo et al. (2015) that contained ferulic acid (0.004–0.085 mg/g DW) and rutin (0.242–0.618 mg/g DW). Based on the current study, phenolic compounds could have an effect on the growth and production of secondary metabolites. It was expected that mature green banana peel would have a greater effect on the growth and secondary metabolites of *M. purpureus* than overripe banana peel. This was confirmed by the higher contents of phenolic compounds in the mature green banana peel than the overripe banana peel.

Table 2 Bioactive compound contents of Hom Thong banana peel at two harvesting periods

Harvesting period	Total phenolic content (mg GAE/g crude extract)	Total flavonoid content (mg CE/ g crude extract)
Mature green	59.74±1.62[a]	27.59±0.89[a]
Overripe	31.14±1.42[b]	6.29±0.08[b]

GAE = gallic acid equivalents; CE = catechin equivalents
Mean ± SD within a column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Table 3 Phenolic compound contents of banana peel at different ripening periods

Phenolic compound	Phenolic compound contents (mg/g dry weight)	
	Mature green	Overripe
Phenolic acid		
Gallic acid	22.37±6.71[b]	17.72±3.90[c]
Caffeic acid	15.80±4.32[d]	15.15±2.78[d]
Coumaric acid	Nd	3.54±2.14[e]
Flavonoid		
Catechin	39.76±2.63[a]	24.29±1.82[b]
Rutin	17.68±2.67[c]	26.55±4.55[a]

Nd = Not detected
Mean ± SD within a column superscripted with different lowercase letters are significantly
($p < 0.05$) different

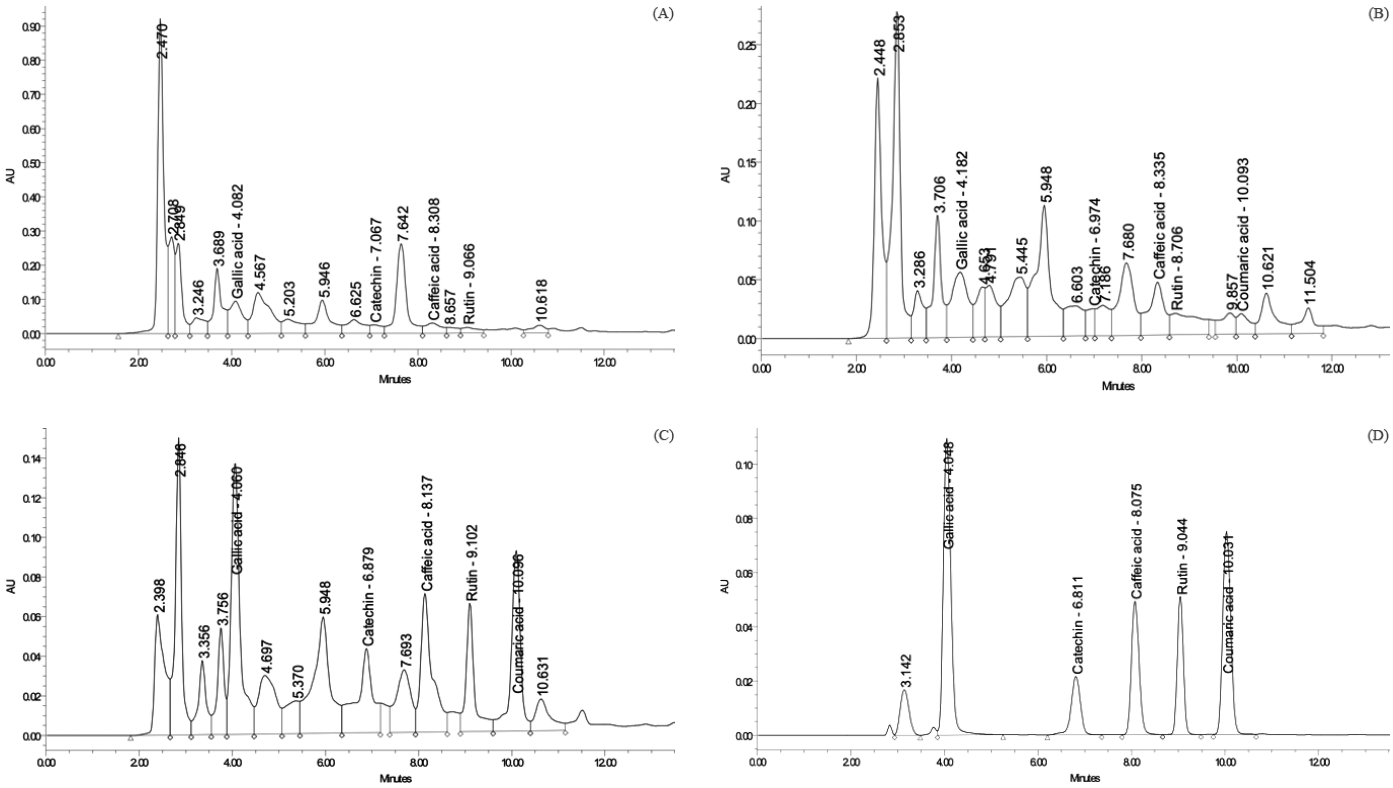

Fig. 1 High performance liquid chromatograms: (A) mature green banana peel extract; (B) overripe banana peel extract; (C) banana peel extract with mixed standards; (D) phenolic compound standards

From a structural viewpoint, isoflavone is potentially effective in reducing the production of citrinin toxin. For example, genistein reduced the production of citrinin by up to 80% due to its structure having an OH group at the positions C5, C4' and C7 (Wang et al., 2020). Other research has indicated that a flavonoid group would reduce the production of the toxin citrinin by *Monascus* sp. (He et al., 2020).

Types of phenolic compounds in inhibition of fungal growth

The purpose of this experiment was to study the effects of the phenolic compounds found in the Hom Thong banana peel on the growth, pigment production and production of citrinin toxin. *M. purpureus* was cultivated on PDB containing standard phenolic substances (gallic acid, caffeic acid, coumaric acid, catechin, rutin) at the same concentration of 1 mg/mL. This concentration was similar to the amount of phenolic compounds found in banana peels. Catechin was the most potent compound in reducing the mycelial growth of *M. purpureus* (96.35%), as shown in Table 4 and was able to inhibit the production of citrinin (92.02%), though coumaric acid was the most potent compound in inhibiting the production of citrinin (98.05%). The ability of rutin to inhibit the production of citrinin was also good (81.12%), whereas gallic acid and caffeic acid were not able to inhibit the mycelial growth of *M. purpureus* and additional mycelial growth was produced. *M. purpureus* produced citrinin toxin during its growth. Under the condition with gallic acid, *M. purpureus* was able to produce citrinin toxin as high as 486.90 ± 48.65 ng/mL, which was double the value of the control group (Table 4). Under the condition with caffeic acid, citrinin toxin production was as high as 273.21 ± 46.57 ng/mL, similar to the PDB medium without any phenolic compound (235.28 ± 15.99 ng/mL), which was higher than the control group. Rutin was found at 44.40 ± 17.76 ng/mL, which was not consistent with the experimental results of Huang et al. (2019b), who reported that flavonoids had an effect on the production of citrinin by *Monascus aurantiacus* Li AS3.4384 (MALA). When 5.00 g/L rutin, α-glucosylrutin or troxerutin were added, the production of citrinin decreased by 29.20%, 54.70% and 40.60%, respectively. Huang et al. (2019a) reported that adding rice powder, inorganic salt or isoflavone at 20 g/L reduced the production of citrinin by *M. aurantiacus* Li AS3.4384 (MALA) to lower than the control (95.98%). In the current study, rutin and catechin reduced the production

Table 4 Effect of banana peel and phenolic compounds on mycelial growth, citrinin production and pigment intensity of *Monascus purpureus* TISTR 3003

Banana peel and phenolic compound	% Inhibition of mycelial growth	Citrinin (ng/mL)	Pigment intensity	
			OD_{500}	OD_{440}
Control	00.00	235.28±15.99^{c}	32.91±00.37^{e}	79.19±01.30^{c}
Mature green (75 mg/mL)	-88.76	278.90±09.33^{b}	62.63±00.16^{a}	118.43±01.90^{a}
Overripe (75 mg/mL)	-92.84	208.83±26.29^{d}	58.57±00.23^{b}	120.03±00.50^{a}
Gallic acid (1 mg/mL)	-58.91	486.90±48.65^{a}	33.54±00.12^{d}	81.35±00.97^{b}
Caffeic acid (1 mg/mL)	-33.79	273.21±46.57^{b}	36.45±00.09^{c}	81.79±00.05^{b}
Coumaric acid (1 mg/mL)	+83.64	4.57±00.73^{g}	3.65±00.13^{h}	10.08±00.10^{e}
Catechin (1 mg/mL)	+96.35	18.77±00.23^{f}	21.23±00.09^{g}	76.62±00.94^{d}
Rutin (1 mg/mL)	+96.05	44.40±17.76^{e}	29.75±00.03^{f}	77.50±01.70^{cd}

OD = optical density; negative value (-) = does not inhibit; positive value (+) = does inhibit
Mean ± SD within a column superscripted with different lowercase letters are significantly ($p < 0.05$) different

of citrinin by 81.12% and 92.02%, respectively. These results were consistent with Wang et al. (2020), who reported that the flavonoid compounds apigenin and kaempferol were able to reduce the production of citrinin by MALA, by 50%, luteolin and quercetin were able to reduce the production of citrinin by 60–67%, while genistein was the most potent by reducing the production of citrinin by up to 80%. It was predicted that the most effective flavonoid compounds in inhibiting the production of citrinin toxin should have a chemical structure including a benzyne ring at the position of C3 and have OH groups at positions C5, C4 and C7, which was consistent with the structure of rutin and catechin, where OH groups are located at position C5, C4' and C7 and were able to reduce the production of citrinin by up to 80%. This result disagreed with Wang et al. (2020). In the current study, *M. purpureus* was able to grow and produced more pigment than the control without phenolic compounds. This was most likely because of the different strains of fungi and the type of medium (PDB) used in the cultivation, whereas Wang et al. (2020) used rice powder and other substances, including sources of carbon, nitrogen and minerals (Huang et al., 2019a).

Experiments on the effect of Hom Thong banana peel powder in the culture medium on the production of pigment and citrinin by *M. purpureus* in PDB were conducted using two types of Hom Thong banana peels (mature green and overripe) as substrates in the cultivation, without promoting the production of citrinin by *M. purpureus* in the cultivation. When banana peel powder was added at 75 mg/mL of PDB culture medium, citrinin was produced in the overripe banana peel powder by *M. purpureus* at 208.83 ± 14.58 ng/mL, which was lower than for the mature green banana peel powder. In contrast, using PDB without banana peel powder, *M. purpureus* produced citrinin at a higher level (235.28 ± 15.99 ng/mL), as shown in Fig. 2. This was consistent with the findings of Panda et al. (2015), who investigated the production of citrinin by *Penicillium citrinum,* extracted from peppermint leaves. All concentration levels of extracts from peppermint leaves (0.21 µg/mL, 0.42 µg/mL, 0.63 µg/mL, 0.84 µg/mL) reduced the production of citrinin. At the highest concentration of 0.84 µg/mL, the production of citrinin was reduced by up to 73% compared to the control without extracts added. It was predicted that the contents of phenolic compounds in the extracts from peppermint leaves were high, which led to a significant reduction in citrinin production. However, in the current experiment using banana peel powder with no extract, the concentration of phenolic compounds was low. Perhaps the quantity of Hom Thong banana peel powder added to the PDB

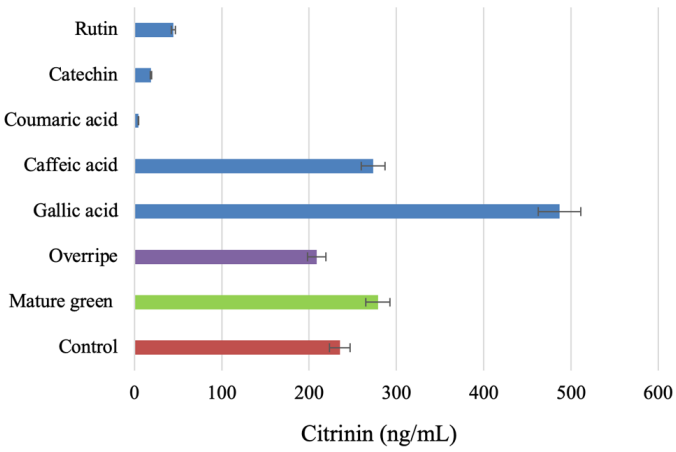

Fig. 2 Effects of Hom Thong (mature green and overripe) banana peel at 75 mg/mL and five different phenolic compounds at 1 mg/mL on citrinin production by *Monascus purpureus* TISTR 3003 cultivation in potato dextrose broth at 25°C for 10 d; Error bars indicate ±SD

was too low, as even though overripe banana peel powder was added up to 75 mg/mL, the reduction in citrinin production was not significantly lower, whereas the extract from peppermint leaves was effective at concentrations up to 1,000 times lower. The results suggested that phenolic compounds from Hom Thong banana peel may affect the growth and production of citrinin toxin by *M. purpureus*. In this experiment, adding banana peel powder into the PDB medium not only reduced the production of citrinin, but it also helped to promote the growth of *M. purpureus*. Thus, overripe banana peel has potential as a substrate in the cultivation of fungi in order to study the characteristics of important compounds from the cultivation of *M. purpureus*. The study of the effects of Hom Thong banana peel powder on the production of pigment in PDB medium indicated that the quantity of banana peel affected the production of pigment. The proportion of banana peel powder in PDA affected not only the growth of *M. purpureus*, but also when added to the PDB, as well as the amounts of red and orange pigments produced. When these two types of banana peel powders were added to the culture medium at 75 mg/mL in PDB, the pigment intensity was measured at wavelengths of 500 nm and 440 nm, which are the best for absorbance by *M. purpureus* as red and orange colors, respectively (Chen et al. 2017). Mature green banana peel powder promoted better production of red pigment by *M. purpureus* at a wavelength of 500 nm than overripe banana peel powder, whereas there was no difference between the two types of banana peel for the production of orange pigment at a wavelength of 440 nm (Table 4).

From this experiment, phenolic compounds affected the growth and production of citrinin by *M. purpureus*. Even though mature green banana peel promoted better production of pigment than the overripe banana peel, after the content of phenolic compounds in the banana peel was studied, the phenolic compounds in Hom Thong banana peel differed as follows: the overripe banana peel had a coumaric acid content of 3.54 ± 2.14 µg/g DM, which was not present in the mature green banana peel. This affected the pigment production by *M. purpureus* as coumaric acid does not promote pigment production (Fig. 3). The pigment intensity of the PDB, when mixed with coumaric acid at wavelengths of 500 nm and 440 nm, had low values at 3.65 ± 0.13 unit/mL and 10.08 ± 0.10 unit/mL, respectively, whereas the control (PDB) had pigment intensities of 32.91 ± 0.37 unit/mL and 79.19 ± 1.30 unit/mL, respectively (Table 4). Inhibition of mycelial growth affected the pigment production and the production of citrinin in the case of coumaric acid, but this did not occur with catechin as pigment was still produced. In summary, regarding pigment production and the production of citrinin toxin by *M. purpureus* in PDB mixed with banana peel powder and different types of standard phenolic compounds, the findings indicated that inhibition of the growth and citrinin production from banana peel would result from the phenolic compounds in the banana peel. Overripe banana peel had a lower total phenolic content than mature green banana peel. Thus, when overripe banana peel powder was added, there was better growth of *M. purpureus* than with mature green banana peel powder. In addition, the overripe banana peel powder led to lower production of citrinin toxin by *M. purpureus* than with mature green banana peel powder. When the experiment was conducted on fungal cultivation in PDB with the addition of different types of phenolic compounds, the pigment production and citrinin production depended on the type of phenolic compounds used. However, using only one type of compound in the experiment produced a different result for using banana peel that contains many different compounds. This experiment also showed that the phenolic compounds in Hom Thong banana peel affected the growth and production of citrinin. Using Hom Thong banana peel as substrate in the cultivation of *M. purpureus* instead of rice, affected the growth and production of secondary metabolites. The next results cover the factors affecting the cultivation of *M. purpureus* using Hom Thong banana peel as substrate, to identify the period of cultivation and the type of banana peel that were most suitable in the cultivation of *M. purpureus*.

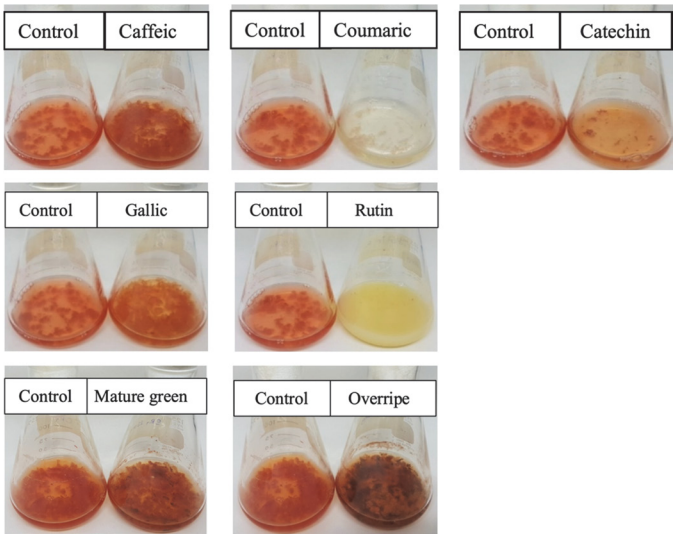

Fig. 3 Growth of *Monascus purpureus* TISTR 3003 cultivated using Hom Thong overripe banana peel and different phenolic compounds as substrates

Study of factors affecting levels of pigments and lovastatin in cultivation of Monascus sp.

Two factors in the cultivation of *M. purpureus*, namely, mature green banana peel and overripe banana peel, were studied using four different incubation times (24 d, 28 d, 32 d, 36 d) at pigment intensity wavelengths of 500 nm and 440 nm (red pigment and orange pigment, respectively). The ripening stage, incubation time and interaction between the two treatments were significant for red pigment and orange pigment. Hom Thong mature green banana peel at an incubation time of 28 d produced significantly greater pigment production than with overripe banana peel at a wavelength of 500 nm (414.67 ± 9.77 unit/g DW and 319.81 ± 0.62 unit/g DW, respectively) and at a wavelength of 440 nm (327.36 ± 7.87 unit/g DW and 275.51 ± 5.30 unit/g DW, respectively). The fact that mature green banana peel promoted better pigment production was consistent with the aforementioned result because the overripe banana peel contained coumaric acid which reduces the production of pigment. However, the cultivation of *M. purpureus* in broth to which banana peel powder had been added resulted in a lower pigment intensity than that of cultivation using solid state fermentation (SSF), as shown in Table 5. This was due to the cultivation medium (PDB). It has been reported that for *M. purpureus* grown in broth, secondary metabolites would be inhibited within the mycelia of *M. purpureus*; however, when cultivation was done using SSF, secondary metabolites would be released outside the cells of the mycelium (Zhang et al., 2015), resulting in the pigment intensity using PDB being lower than for cultivation using SSF. Thus, Hom Thong mature green banana peel was used as substrate to promote better pigment production. The pigment intensity at a wavelength of 500 nm was 414.67 ± 9.77 unit/g DM and at a wavelength of 440 nm was 327.36 ± 7.87 unit/g DM (Table 5). Cultivation of *M. purpureus* in overripe banana peel significantly delayed pigment production during the incubation times of 32 d and 36 d. When corn cob was used as substrate, cultivation of *M. purpureus* as a source of carbon and glycerol as a source of nitrogen, the pigment intensities at wavelengths of 500 nm and 400 nm were 108.02 units/mL and 133.77 units/mL, respectively (Embaby et al., 2018), which were lower than from using banana peel as a source of carbon in the cultivation of *M. purpureus*. Rice is another type of substrate and was used by Liang et al. (2018) as substrate in cultivation and the levels of pigment intensity at wavelengths of 410 nm, 465 nm and 505 nm were 8,539 ± 426 units/g, 8,470 ± 332 units/g and 7,667 ± 321 units/g, respectively. These levels were 22 times higher than for Hom Thong banana peel. However, at the same time, by using rice as substrate, the level of citrinin toxin was as high as 49.17 ± 2.64 µg/g. In the current study in which Hom Thong banana peel was used as substrate, no citrinin toxin was produced for an incubation period of 36 d because the Hom Thong banana peel has phenolic compounds as part of its composition and they have been reported as effective in reducing the production of citrinin toxin in *M. purpureus*. For example, eugenol is able to control the production of citrinin by *Penicillium citrinum* (Orozco and Kilikian, 2008) and caffeic acid is able to inhibit the growth and synthesis of aflatoxin and ochratoxin by *Aspergillus flavus* (Palumbo et al., 2007). Caffeic acid, catechin and coumarin are able to inhibit the synthesis of ochratoxin T-2 and HT-2 by *Aspergillus* sp. and *Fusarium* sp. (Ferruz et al., 2016).

Table 5 Lovastatin and citrinin production and pigment intensity by *Monascus purpureus* cultivated under different ripening stages of Hom Thong banana peels and incubation time in SSF

Ripening stage	Incubation time (d)	Lovastatin (mg/g)	Citrinin (ng/g)	Pigment intensity (unit/ g DW)	
				OD_{500}	OD_{440}
Mature green	24	201.34±04.92^{g}	Nd	348.20±9.21^{b}	309.51±08.07^{b}
	28	378.59±19.07^{f}	Nd	414.67±9.77^{a}	327.36±07.87^{a}
	32	467.98±06.22^{d}	Nd	340.95±8.62^{b}	301.49±00.87^{b}
	36	412.64±09.24^{e}	21.20	302.05±0.09^{d}	272.15±01.42^{c}
Overripe	24	479.27±07.18^{d}	Nd	312.89±3.48^{c}	223.31±14.56^{d}
	28	752.16±14.30^{b}	Nd	319.81±0.62^{c}	275.51±05.30^{c}
	32	861.91±04.45^{a}	Nd	300.15±0.83^{d}	275.35±04.98^{c}
	36	689.65±10.66^{c}	37.32	276.27±6.28^{e}	273.54±01.99^{c}

OD = optical density; Nd = not detected

Mean ± SD within a column superscripted with different lowercase letters are significantly ($p < 0.05$) different

Therefore, it was predicted that *M. purpureus* using Hom Thong banana peel and SSF would produce citrinin toxin because of the phenolic compounds that are components in Hom Thong banana peel and these affect the growth, pigment production and production of citrinin. The phenolic compounds used in the current experiment had different effects on the inhibition of mycelial growth and the production of citrinin and pigment, depending on the particular type of phenolic compound used. However, the banana peel contained several types of phenolic compounds and this resulted in different effects on the inhibition and promotion of growth and on the production of toxin. Such a result differed from using only one particular pure phenolic compound in the experiment. Furthermore, Hom Thong banana peel has other types of phenolic compounds that were not studied in the current research.

The study of factors in cultivation of *M. purpureus* used two stages of banana as substrate (mature green and overripe) with the moisture content starting at 55% and four incubation periods (24 d, 28 d, 32 d, 36 d). The ripening stage, incubation time and interaction between the two treatments had a significant effect on lovastatin production. In the current study, the maximum levels of lovastatin and citrinin from the cultivation of *Monascus* sp. were based on incubation for 32 d, with the level of lovastatin being higher from the overripe banana peel than for the mature green banana peel. There was a low level of lovastatin produced using incubation of 24 d with mature green banana peel as substrate because *M. purpureus* has a longer incubation time than the overripe banana peel perhaps because there were higher levels of phenolic compounds in the mature green banana peel than in the overripe banana peel. The highest level of citrinin was produced after incubation for 36 d (Table 5). Other research studies in which lovastatin was produced by *Monascus* sp. using rice as the substrate, reported contamination of citrinin (Singgih et al., 2014; Liang et al., 2018). In addition, it was found that when rice was used as the substrate, the concentration of lovastatin was four times lower than when banana peel was used. However, the difference may have been due to the type and variety of fungus used in the cultivation and the shorter incubation time. Using other types of fungus, such as *Aspergillus terreus*, in the study of lovastatin production and using wheat bran as the substrate resulted in good production of lovastatin. For example, with glucose at 3% as a source of carbon, *Aspergillus terreus* produced lovastatin levels as high as approximately 5,000 µg/g of substrate (Kamath et al., 2015), which was higher than the level obtained from cultivation of *M. purpureus* using Hom Thong banana peel as substrate. However, using *Aspergillus terreus* would result in brown pigmentation so that additional steps of purification would be needed that would add to the cost of production.

The study considered the effects of two factors in the cultivation of *M. purpureus*, (either mature green or overripe stages of Hom Thong bananas) and the effect of incubation time on pigment, lovastatin and citrinin production. The results showed that cultivation factors in the fermentation by *M. purpureus* significantly affected lovastatin production when overripe banana peels were used as substrate in the fermentation process and incubated for 32 d, producing the highest lovastatin content. The optimum conditions to produce pigments were using mature green banana peels as substrate in the fermentation process and incubating for 28 d. It was found that Hom Thong banana peel effectively inhibited the production of citrinin by *M. purpureus*. Mature green banana peel promoted pigment production better than overripe banana peel. However, overripe banana peel promoted lovastatin production better than mature green banana peel. Therefore, it would be more beneficial to use Hom Thong overripe banana peel in the cultivation of *M. purpureus* because lovastatin has a higher market value than pigment.

In conclusion, Hom Thong banana peel has phenolic compounds which affect the growth and production of secondary metabolites of *M. purpureus*. Hom Thong banana peel can be used as a carbon source in the growth of *M. purpureus*. Hom Thong banana peel can also be used as a good substrate for pigment and lovastatin production with a minimum citrinin content by *M. purpureus*. There should be further study on the effects of growth and the production of secondary metabolites of *M. purpureus* by important substances in Hom Thong banana peel.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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