

Study on durian seed as a new substrate for angkak production

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Abstract: A study was carried out to examine the potential of durian seed as a new substrate of *Monascus* sp. for angkak production. The durian seeds were analyzed for their proximate composition and starch content. After pretreatment, different volumes of distilled water were added to sterile small size cut of durian seeds to set the initial moisture content of 58%, 59%, 60%, 61% and 62%, inoculated with *Monascus* sp. KJR2 culture and were incubated for 14 days at room temperature (30°C). The fermented-durian seeds were dried and analyzed for the yellow, orange and red pigments. The durian seeds contain 54.90% moisture, 1.58% ash, 3.40% protein, 1.32% fat and 18.92% starch. The results indicated that initial moisture content significantly affect the pigments content. Water-soluble yellow, orange and red pigments contents were in the ranges of 6.01 to 11.17 AU/g, 3.82 to 8.52 AU/g and 3.57 to 8.11 AU/g, respectively. Ethanol-soluble yellow, orange and red pigments contents were in the ranges of 1.09 to 3.86 AU/g, 0.51 to 2.51 AU/g and 0.72 to 3.73 AU/g, respectively. The substrate with initial moisture content of 60% was the most suitable substrate for angkak production, yielding Monacolin K of 50 mg/kg. The durian seed has good potentiality to be used as a new substrate for angkak production and more researches are needed to increase the monacolin K content and to evaluate the durian seed angkak functionalities and safety.

Keywords: Durian seed, angkak, *Monascus* sp., moisture content, monascus pigments

Introduction

Angkak, *Monascus*-fermented rice, has long been known and extensively consumed by people in Asian countries. The *Monascus* sp. produces 3 pigment groups i.e. red pigment group (rubropunctamine and monascorubramine), orange pigment group (rubropunctatin and monascorubrin) and yellow pigment group (monascoflavin and ankaflavin). It has been used to color and preserve foods and beverages such as Chinese cheese, bagoong, wine, tofu, sake, miso, pork, sausage and fish (Lin and Demain, 1992; Lin and Demain, 1993; Kumar and Sinha, 2004; Dufosse *et al.*, 2005; Mapari *et al.*, 2005; Blanc *et al.*, 2005). In folk Chinese traditional medication, angkak has been used for cardiovascular system therapy, which has been described in ancient Chinese pharmacopeia, Ben Cao Gang Mu, composed by Shi-Zen Li in 1518-1593 (Lin *et al.*, 2008). Many researchers found that angkak contains monacolin K, which can reduce blood cholesterol. Recently,

other new metabolites have been reported and were found to have positive effects to promote health such as lowering blood glucose and prevent cancer cells (Rajasekaran and Kailavani, 2011; Shi *et al.*, 2011).

Traditionally, angkak is produced by solid state fermentation with rice as the substrate. Many studies showed that it grows in a wide variety of natural substrates i.e. corn, cassava, wheat, potato and adlay (Yongsmith *et al.*, 1998; Ganrong *et al.*, 1998; Carvalho *et al.*, 2007; Pattanagul *et al.*, 2008), various agro-industrial residues i.e. rice bran, wheat bran, cassava bagasse, and jack fruit seed (Dufosse *et al.*, 2005; Babitha *et al.*, 2006).

Durian (*Durio zibethinus* Murr) seed is an agro-industrial residue which is usually discarded. Durian, usually called as 'King Fruit', is a valuable tropical fruit in some Asian countries. In 2010, production of durians in Indonesia was 492,139 tons (Badan Pusat Statistik, 2011). Seeds make up around 5-15% of the total fruit mass. According to Brown (1997), fresh durian seeds contain high moisture (51.5%), carbohydrate (43.6%) and protein (2.6%). But, there

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is no research on the potentiality of durian seed as a substrate for angkak production. The objective of the research was to study the durian seed as a new substrate for angkak production.

Materials and Methods

Durian seeds

Durian seeds (variety Manalagi) were obtained from local durian seller. Durian seeds were stored in a freezer (-4°C) until used. Proximate and starch content analysis of durian seeds were conducted by standard methods of AOAC (1990).

Culture and starter preparation

Monascus sp. KJR2 was obtained from Center for Food and Nutrition Research, Widya Mandala Surabaya Catholic University. It was maintained on Saboraud's Dextrose Agar (SDA) slant, preserved at 4°C and subcultured monthly. After *Monascus* sp. KJR2 was grown on SDA slants at room temperature (30°C) under static conditions for 14 days, 10 mL of sterile distilled water was added and the spores were scraped under aseptic conditions. 0.1 mL of the spore suspension was inoculated into Saboraud's Dextrose Broth (SDB) and then was incubated at room temperature (30°C) for 10 days. It was used as starter to produce monascus pigments on sterilized durian seed.

Durian seeds pretreatments

Durian seeds were pretreated to remove the sticky mucus with different conditions: 1) boiling in a CaCO₃ solution of 5% w/v for 10 min; 2) soaking in CaCO₃ solution of 5% w/v overnight; and 3) control (no pretreatment). After pretreatment, durian seeds were cut into small size of 0.3 cm x 0.3 cm x 0.5 cm, sterilized, and was observed for their color and mucus, qualitatively. The sterile small cut durian seeds were inoculated with *Monascus* sp. KJR2 starter culture, incubated for 7 days and was observed qualitatively for mold growth and color.

Solid-state fermentation (SSF)

Durian seeds were boiled in a CaCO₃ solution of 5% w/v for 10 min to remove the mucus, and then peeled the seed coat, cut into small size of 1 cm x 1 cm x 1 cm. A 50 g of small cut durian seed was transferred into 300 mL flask, added with distilled water to set the moisture content of substrate of 58%, 59%, 60%, 61% and 62%. The contents of the flasks were mixed thoroughly, autoclaved at 121°C for 15 min, then left to cool to room temperature, inoculated with the spore suspension of *Monascus* sp. KJR2 and incubated at room temperature (30°C) for 14 days in

static conditions (with manual shaking daily). Red mold durian seed were dried in an oven at 45°C for 24 hours, ground and was analyzed for the water soluble pigments, ethanol soluble pigments and Monacolin K.

Ethanol soluble pigments analysis

Ethanol soluble pigments were analyzed according to Babitha *et al.* (2006) with slight modification. One g of fermented matter was transferred in a 250-mL conical flask and mixed with 90% ethanol at a ratio of 5 mL of distilled water per gram of fermented matter on dry basis. The content was mixed by shaking at 200 rpm for 1 hour, left to stand for 15 min, and then filtered through Whatman No 1 filter paper. The filtrate was measured using spectrophotometer (Shimadzu, UV 1601) at 392 nm for yellow pigment, 470 nm for orange pigment and 501 nm for red pigment. Pigment yield was expressed as Absorbance at corresponding wavelength per gram of dry substrate (AU/g).

Water soluble pigments analysis

Water soluble pigments were analyzed according to Carvalho *et al.* (2007) with slight modification. One g of fermented matter was transferred into a 250-mL conical flask and mixed with distilled water at a ratio of 5 mL of distilled water per gram of fermented matter on dry basis. The content was shaken at 200 rpm for 1 hour, left to stand for 15 min, followed by centrifugation at 5000 rpm at 30°C for 15 min, and then filtered through Whatman No 1 filter paper. The filtrate was measured by using spectrophotometer (Shimadzu, UV 1601) at 400 nm for yellow pigment, 470 nm for orange pigment and 501 nm for red pigment. Pigment yield was expressed as Absorbance at corresponding wavelength per gram of dry substrate (AU/g).

Total mold analysis

Total mold analysis was carried out according to Permana *et al.* (2003). One g of fermented material was mixed with 9 ml of 0.1% sterile peptone water. One mL of homogenized mixture was aseptically transferred into 9 mL of sterile peptone water in a glass tube and was serially diluted to 10⁻², 10⁻³ and 10⁻⁴ dilution factors. One mL of each dilution were transferred into duplicated petri dishes followed by the addition of 12-15 mL of SDA (50 ± 1°C) into each plate, mixed thoroughly and after solidification of the agar, they were incubated for 72 hours at 30°C to allow growth of the mold to be observed.

Monacolin K analysis

Monacolin K in the product of the optimum initial moisture content was analyzed according to

Wang *et al.* (2006) with slight modification. One gram of product was extracted with 5 mL of ethyl acetate in shaking water bath at 70°C for 1.5 hours, and then filtered through filter paper. The filtrate was dried under vacuum condition, and then added with 1 mL of acetonitrile, followed by filtration through a 0.45 µm pore size PTFE filter and analyzed by High Performance Liquid Chromatography (HPLC, model LC-20A Prominence, Shimadzu, Japan). Chromatographic separation was conducted on a Shim-Pack ODS C18 column (250 mm x 4.6 mm i.d.). Acetonitrile-phosphoric acid (0.5%), 65:35 v/v was used as the mobile phase. The eluent was pumped at a flow rate of 1.0 mL/min. Monacolin K was detected by SPD 20-A UV-Vis detector at 238 nm. Monacolin K from Sigma-Aldrich was used as standard.

Data analysis

The data were analyzed using analysis of variance (ANOVA) at $\alpha = 5\%$. If the ANOVA test results indicate a significant effect, this was followed by Duncan's Multiple Range Test (DMRT) at $\alpha = 5\%$ to determine the level of treatment that gives a significant difference.

Results and Discussion

Durian seeds pretreatments

Physically, durian seeds contain white endosperm covered by brown seed coat. Table 1 showed the observation results on small cut durian seeds prior and after fermentation with different pretreatments. Pretreatments by boiling in CaCO₃ solution was found to be the most suitable in removing the mucus, which promote the browning process during autoclaving and significantly inhibit the growth of *Monascus*. Too much mucus causes higher adhesiveness among the small cut durian seeds and can reduce surface area for growing of the mold, inhibit oxygen transfer and nutrition diffusion. Though CaCO₃ solution reduces mucus significantly, no growth was observed on the small cut durian seeds. After autoclaving, the small cut durian seeds become too firm, which may be due to the reaction between calcium ion and substances in the seeds. These reactions may contribute to the lack of growth of the mold on the substrate. Based on the results, boiling in CaCO₃ 5% solution for 10 min was used as pretreatment method on durian seed substrate for solid state fermentation.

Chemical composition of durian seeds

The durian seeds contain 54.90% moisture, 1.58% ash, 3.40% protein, 1.32% fat and 18.92% starch. Those results are comparable to that reported by Brown (1997). Naturally, *Monascus* sp. grow well on

Table 1. Observed appearance of durian seeds with different pretreatment

Pretreatment	Before fermentation		After fermentation (7 days)	
	Mucus	Brown intensity	Mold growth	Red intensity
Boiling in CaCO ₃ solution, 10 minutes	+	+	+++	+++
Soaking in CaCO ₃ solution, overnight	-	++	-	-
No pretreatment	+++	++++	+	-

Table 2. Total mold of 14 days fermented durian seeds

Moisture content	Total mold (cfu/g)
Control*)	3.43 × 10 ⁶
59 %	4.20 × 10 ⁶
60 %	5.67 × 10 ⁶
61 %	5.27 × 10 ⁶
62 %	4.70 × 10 ⁶

Note: *)control is no water addition (moisture content of 58%)



Figure 1. Durian seeds after 1 day, 7 days and 14 days fermentation

various solid substrates with various moisture content (26-70%) (Sato *et al.*, 1983; Lotong and Suwanarit, 1990; Babitha *et al.*, 2006). Moisture content is a critical factor in solid state fermentation. The starch content of durian seeds was 18.92%, which is half the jackfruit seeds of 36.7% dry basis (Babitha *et al.*, 2006). The chemical composition reflects that durian seeds have a potential as substrate for angkak production. The potentiality was shown in Figure 1, where *Monascus* sp. KJR2 grew well and produced red pigments during fermentation.

Effect of initial moisture content on pigments production

Generally, *Monascus* sp. KJR2 grew well on durian seeds at various initial moisture content. Table 2 shows total mold count on fermented durian seeds at various initial moisture content. Yellow, orange and red pigments were measured spectrophotometrically at 392 nm, 470 nm and 501 nm. Water-soluble and ethanol-soluble pigments produced by *Monascus* sp. KJR2 on durian seeds at various initial moisture contents were shown in Figures 2 and 3. *Monascus* sp. KJR2 produced considerable amount of yellow, orange and red pigments. Water-soluble yellow, orange and red pigments contents were in the ranges of 6.01 to 11.17 AU/g, 3.82 to 8.52 AU/g and 3.57 to 8.11 AU/g, respectively. Ethanol-soluble yellow, orange and red pigments contents were in the ranges of 1.09 to 3.86 AU/g, 0.51 to 2.51 AU/g and 0.72 to 3.73 AU/g, respectively.

Addition of distilled water into durian seeds

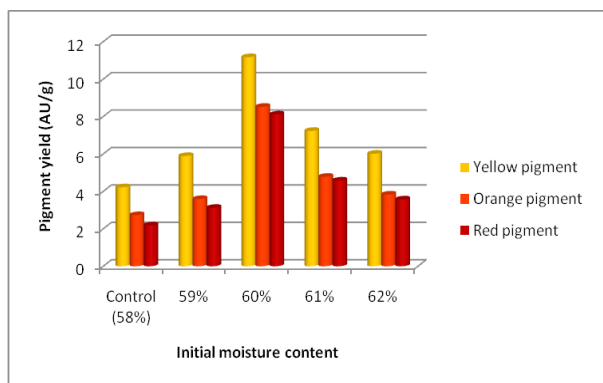


Figure 2. Effect of initial moisture content on water soluble pigments production

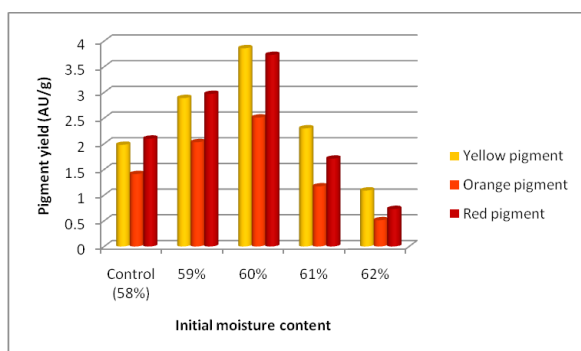


Figure 3. Effect of initial moisture content on ethanol soluble pigments production

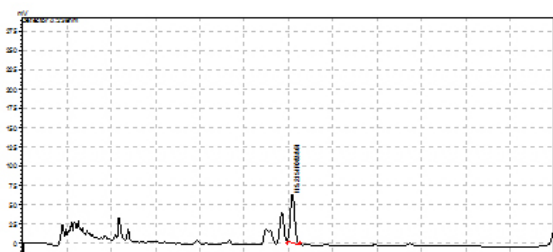


Figure 4. Chromatogram of sample of monascus-fermented durian seed at initial moisture content of 60%

substrate significantly increased pigments production by *Monascus* sp KJR2. As reported by Ganrong *et al.* (2005), moisture is a key parameter to control the growth of microorganisms and metabolite production in solid-state fermentation of *Monascus* red rice. As for fermentation of *Monascus* red rice in Erlenmeyer flasks, the methods to control the moistures of the solid-state materials are usually done by addition of suitably initial water to the raw materials. Water plays an important role in almost all biochemical reactions in fungi cells. Sato *et al.* (1983) reported that optimum initial moisture content is favorable for pigmentation and liberating glucose due to glucoamylase activity.

In this study, increasing the initial moisture contents up to 60% significantly increased pigments

production. Lower moisture content will not be sufficient to support the growth and metabolism processes of the fungi. Ganrong *et al.* (2005) reported that the initial moisture content of 53.78%, the substrates were too dry and very poor growth of *Monascus* observed. Thus, increasing initial moisture content up to 64.75% enhanced the growth of the fungi.

However, at higher moisture contents, there is a significantly decrease in the production of pigments. It may probably due to the fact that too much water inhibits the supply of oxygen into fungi cells. Similar observations were reported by other researchers on rice granules. When the moisture content increases, the substrate porosity and uniformity of the rice granule are reduced along with the reduction in O₂ transfer. The optimum initial moisture content favored the mass transfer, intake of oxygen and release of carbon dioxide (Sato *et al.*, 1983; Lotong and Suwanarit, 1990; Ganrong *et al.*, 2005; Babitha *et al.*, 2007).

The ethanol soluble red pigment at initial moisture content of 60% (3.73 AU/g) was higher than that produced at 30°C for 14 days on peanut meal (3.03 AU/g), soybean meal (3.01 AU/gds), and coconut residue (0.59 AU/g), but lower than that produced on corn meal substrate (19.40 AU/g) (Nimnoi and Lumyong, 2011). It is concluded that durian seed are potential as substrate for angkak production with considerable amount of pigments, however the pigments productivity on this substrate need to be improved. Other researchers found that supplementation of carbon and nitrogen sources on agricultural residues such as jackfruit seed, peanut meal, soybean meal, coconut residue and corn meal, increased monascus pigments production (Babitha *et al.*, 2007; Nimnoi and Lumyong, 2011).

Monacolin K

Figure 4 showed the chromatogram of sample of monascus-fermented durian seeds at initial moisture content of 60%. The product contains monacolin K which was detected at time retention of 15.2 min. The content is 50 mg/kg, higher than adlay angkak (14.97-25.03 mg/kg) (Pattanagul *et al.*, 2008), comparable to wheat (broken) and finger millet angkak (60-80 mg/kg) and lower than rice angkak (160-640 mg/kg) (Venkateswaran and Vijayalakshmi, 2010). It is concluded that durian seeds are potential as substrate for angkak production with considerable amount of monacolin K, however the productivity on this substrate need to be improved. Panda *et al.* (2010) found that fermentation process parameters i.e. temperature, fermentation time, inoculum volume

and pH influence the monacolin K production.

Conclusions

The durian seed has good potentiality to be used as a new substrate for angkak production. Initial moisture content of durian seed substrate of 60% was the optimum for *Monascus* sp. KJR2 growth and pigments production, with monacolin K content of 50 mg/kg. More researches to improve the pigments and monacolin K are needed. Further researches on functionalities and safety of durian seed angkak were also needed.

Acknowledgment

Thanks to Directorate General of Higher Education, Ministry of National Education, Republic of Indonesia for the financial support through competitive research Penelitian Hibah Bersaing with contract number of 135/SP2H/PL/Dit. Litabmas/IV/2011 and Program Hibah Kompetensi Institusi Batch II.

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