Simultaneous detection of monacolins and citrinin of angkak produced by \textit{Monascus purpureus} strains using Liquid Chromatography-Mass Spectrometry (LC-MS/MS)

1,2Sulanndari, L., 2,3Utami, T., 2Hidayat, C. and 2,3*Rahayu, E.S.

1Department of Home Economics, Engineering Faculty, Universitas Negeri Surabaya Jl. Ketintang, Surabaya 60231, Indonesia
2Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Jl. Flora No.1 Bulaksumur, Yogyakarta 55281, Indonesia
3Center for Food and Nutrition Studies, Universitas Gadjah Mada, Jl. Teknika Utara, Sleman, Yogyakarta 55281, Indonesia

Abstract

Angkak or red mold rice is produced from the fermentation of white rice using \textit{Monascus purpureus}, which results in the red color of the fermented rice. Angkak has been used as a natural coloring agent for food, spices, and medicine. The active compound in angkak that contributes to lower blood cholesterol is known as monacolin (monacolin K). However, the presence of citrinin, the byproduct of angkak fermentation, needs to be considered as it can cause hepatonephrotoxic mycotoxin. The contents of pigments, monacolins, and citrinin as secondary metabolites depend on the \textit{Monascus} strain and fermentation conditions. This study aims to analyze simultaneously monacolins and citrinin in angkak produced by \textit{M. purpureus} strains using Liquid Chromatography-Mass Spectrometry (LC-MS/MS). The angkak was prepared by fermentation using \textit{M. purpureus} FNCC 6008 and \textit{M. purpureus} JK2. A total of 10^7 \textit{M. purpureus} spores/mL was inoculated into the rice. The fermentation was carried out at room temperature (25-30°C) for 14 days. The detection using LC-MS/MS showed that the monacolin K in angkak from both strains was below LOQ (< limit of quantification). The analysis of citrinin content in angkak showed that JK2 strain produced lower citrinin (1.10±0.021µg/g) compared to FNCC 6008 (3.01±0.072 µg/g). The other monacolins found in angkak from both strains were including dehydromonacolin K, monacolin J (qualitative), and mevastatin. Based on t-test, the amount of both mevastatin and citrinin in angkak produced by two different strains were significantly different. In contrast, the amount of dehydromonacolin K in both angkak was comparable. The simultaneous detection result of LC-MS/MS could determine the choice of \textit{Monascus} strains quickly. JK2 strain was considered as safe, thus it could be chosen to be applied to food products.

1. Introduction

Angkak or red mold rice (RMR) is a product of \textit{M. purpureus} fermentation via solid-state fermentation using rice as the primary media. There are many names to refer to angkak, in China and Taiwan angkak is called “Hong Qu”, “Hon-Chi”, “Anka”, or “Ang-kak”; whereas the Japanese people use the name of “Beni Koji” or “red Koji”. In America and Europe angkak is called as “red rice”, “red mold rice” or “red Chinese rice”. However, the name red yeast rice (RYR) is often used in many publications and commercial products even though it is not an appropriate name for filamentous fungi (Wang and Lin, 2007; Song eta., 2019).

\textit{Monascus} can produce beneficial secondary metabolites, such as pigments (food-grade colorants), monacolins (cholesterol-lowering agents), γ-butyric acid (an antihypertensive substance), dimerumatic acid (an antioxidant) (Chung et al., 2009; Chen et al., 2015; Song et al., 2019). The red pigment of angkak is mainly used as a natural coloring for food, but it can also be used as a preservative in meat and fish products (Wang and Lin, 2007; Chen et al., 2015), cheese (Devi and Meera, 2015) as well as an agent to increase platelets in dengue hemorrhagic fever (Danuri, 2008; Ristiarini et al., 2007; Wang et al., 2007; Song et al., 2019).
Monacolins can be used as a cholesterol-lowering agent (Campbell and Vederas, 2010; Heinz et al., 2016) by inhibiting the enzyme of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase in cholesterol biosynthesis (Song et al., 2012; Heinz et al., 2016). Approximately 14 structural forms of monacolin have been identified from the genus of Monascus, namely: monacolin K (monacolin K lactone form; monacolin K acid form), monacolin J, monacolin J acid form, monacolin L, monacolin L acid form, monacolin M, monacolin M acid form, monacolin X, monacolin X acid form, and dehydromonacolin K, dihydromonacolin L, compactin, 3α hydroxy 3,5 dihydromonacolin L (Li et al., 2004; Avula et al., 2014).

Citrinin was reportedly detected in various M. purpureus fermented products (Vuković et al., 2017). Citrinin, a mycotoxin which has harmful effects on the function and structure of the kidney and is able to modify liver metabolism (Mornar et al., 2013; Ji et al., 2015). The maximum level of citrinin in red yeast rice-based food supplements is 2000 µg/kg (2 ppm) (Commission Regulation (EU) No 212/2014 of 6 March 2014). The presence of citrinin in food products needs to be considered to ensure product safety.

The content of secondary metabolites of angkak depends on the Monascus strain and fermentation conditions. Detection of the amount of monacolin K and citrinin has been carried out on various Monascus fermented products in the form of angkak and food supplements. Detection of monacolin K (Li et al., 2004; Song et al., 2012; Di Donna et al., 2018) and citrinin (Ji et al., 2015; Vuković et al., 2017; Vuković et al., 2019) can be carried out individually or simultaneously (Nigović et al., 2013; Mornar et al., 2013; Avula et al., 2014). Chromatographic techniques are often used in the detection of monacolin K and/or citrinin (Avula et al., 2014). Although the chromatographic technique is sensitive and selective (Nigovic et al., 2013), it is time-consuming and requires expensive equipment and solvents. Chromatographic techniques that have been developed to simultaneously detect monacolin K and citrinin that are fast and accurate include: chromatography electromagnetic capillary chromatography (Nigovic et al., 2013), liquid chromatography with array diode detectors (DAD) and/or with mass spectrometry (MS) (Mornar et al., 2013) and liquid chromatography-DAD-QtoF-MS (Avula et al., 2014).

Fast detection of monacolin K and/or citrinin in Monascus fermentation products have been carried out for screening products from adulteration, including labels that do not match the contents of product (Li et al., 2017a). Simultaneous detection of monacolin K and citrinin in angkak produced by M. purpureus strains has not been widely reported. This study aimed to analyze simultaneously monacolins and citrinin in angkak produced by M. purpureus strains using Liquid Chromatography-Mass Spectrometry (LC-MS/MS).

2. Materials and methods

2.1 Microorganism

There were three strains of M. purpureus used in this study. The strain of M. purpureus FNCC 6008 was purchased from the collection of Food and Nutrition Collection Center (FNCC), Universitas Gadjah Mada Indonesia; M. purpureus JK2 strain was obtained from Widya Mandala University, Surabaya, Indonesia that was isolated from angkak at a pharmacy shop in Jakarta; and M. purpureus HD-CC 001 was obtained from the collection of Microbiology Laboratory, School of Pharmacy, Bandung Institute of Technology. The culture strains were inoculated into potato dextrose agar (PDA; Merck) slants, and then incubated at room temperature (25-30°C) for 14 days. Spore suspensions were prepared by the addition of sterilized water into the grown culture on PDA agar slant. The spore concentration was 10^7 spores per mL as counted by using haemocytometer.

2.2 Solid-state fermentation

Solid-state fermentation was conducted by inoculating M. purpureus culture into the rice. The IR 64 rice was purchased from Giant supermarket in Yogyakarta. The rice was soaked in water (1:1) for 24 hours. After soaking the rice was rinsed and drained. A total of 100g of rice was put into a 500 mL erlenmeyer and sterilized at 121°C for 15 mins and then cooled to room temperature. The sterilized rice was inoculated with 10mL spore suspension of M. purpureus (10^7 spores/mL). After cultivation at room temperature (25-30°C) for 14 days, the angkak (fermented rice) was dried with cabinet dryer at 40°C for 15 mins and then cooled to 25°C. The dried angkak was blended to a fine powder before extraction.

2.3 Chemicals

Monacolin K (lovastatin) and citrinin standard were ordered from Sigma-Aldric RTC, WY, USA. Acetonitrile gradient grade and formic acid were purchased from Germany. Water used from the purification process (Evoqua).
2.4 Sample extraction

The sample extraction and analysis using LC-MS/MS followed Avula et al. (2014) and Di Dona et al. (2018) with modification. Approximately 0.04 g of angkak powder was extracted using 10 mL of acetonitrile in a sonicator (Branson 2200, USA) at 30°C for 30 mins. The obtained mixture was heated with a waterbath at 60°C for 1 hr and then centrifuged (Zenith Lab, LC-04S, China) at 3500 rpm for 10 min. The amount of 0.01 g adsorbent (MgSO4: Na-Acetate, modification) was added into 1 mL supernatant and then centrifuged (Hitachi, Japan) at 10000 rpm for 5 mins. It was followed by filtration through nylon 0.2 µm (Agilent, USA). The filtrate (2µL) was injected into the LC-MS/MS.

2.5 Mass spectrometry condition

The sample analysis was carried out using LC-MS/MS, Thermo Scientific UHPLC instrument, Accela LC type 1250 (Thermo Fisher Scientific, USA). The chromatographic separation used Hypersil Gold (50 mm x 2.1 mm x 1.9 µm) column. Solvent A consisted of 0.1% formic acid in aquabidest and solvent B consisted of 0.1% formic acid in acetonitrile. The flow rate was set at 300 µl/min and the sample injection volume was 2µl. The linear gradient with the adjustment of the mobile phase was as follows: 0-0.6 min, 75% A; 0.6-3.0 min, 90% B; 3.0-4.0 min, 90% B; 4.0-4.5 min, 25% B and 4.5-6.0 min, 75% A. The column was set at 30°C, and the autosampler compartment was set to 16°C.

The use of MS/MS Triple Q (quadrupole) TSQ Finnigan with ESI (electrospray ionization) as ion sources was controlled by TSQ Tune software which was operated in a positive ion mode. ESI ionization sources was controlled by TSQ Tune software which was operated with ESI (electrospray ionization) as ion source. Quantum Access Max mass spectrometers from Thermo Finnigan with ESI as ion source were controlled by TSQ Tune software. The autosampler compartment was set to 16°C.

Determination of quantity used the SRM (selected reaction monitoring) method. Monacolin K molecules were adjusted with precursor ions (m/z) 405, and product ion (m/z) 199 while the citrinin was adjusted with precursor ion (m/z) 251, and the product ion (m/z) 233.

2.6 Color measurement

Color measurement was conducted using chromameter CR 400 (Konica Minolta Co. Ltd., Osaka, Japan). White calibration using the white calibration plate was done before measuring. The ground samples (angkak) were poured in a cuvette. The measurement button was pressed after making sure the ready lamp ON. Measurement was done, and the data was displayed. The measurement was expressed in three parameters of L*, a* value, and b* value. The L* value is a lightness variable which has a value from 0 (black) to 100 (white). The a* indicates redness (+value) or greenness (-value), whereas b* indicates yellowness (+value) or blueness (-value).

2.7 Statistical analysis

The results were statistically analyzed using t-test. The statistical product and service solution (SPPS) statistical software version 22 was used for statistical analysis.

3. Results and discussion

3.1 Growth of Monascus purpureus in PDA medium

M. purpureus spore was grown on PDA medium and it germinated to form branched hyphae. A mass of hyphae is termed mycelium (Manan et al., 2017). In this study, the color development of 3 strains M. purpureus was observed by measuring the colony color in PDA medium during incubation at room temperature (25-30°C) for 14 days.

Table 1. The color change of M. purpureus in PDA medium during incubation at room temperature (25-30°C) for 14 days

| Incubation (days) | Colony color   | Strain | FNCC 6008 | JK2  | HD-CC 001 |
|------------------|----------------|--------|-----------|------|-----------|
| 1                | White          | +      | +         | +    |           |
| 2                | White          | ++     | ++        | +    |           |
| 3                | White          | ++     | +         |      |           |
| 4                | White-yellowish| ++++   | +++       | +    |           |
| 5                | White-yellowish| +++    | +++       | +    |           |
| 6                | White-yellowish| ++++   | +++++     | +    |           |
| 7                | Orange         | +++++  | +++++     | +    |           |
| 8                | Orange         | ++++++ | +++++     | +    |           |
| 9                | Rich orange    | ++++++ | +++++     | +    |           |
| 10               | Rich orange    | +++++++| +++++     | +    |           |
| 11               | Orange-red     | ++++++ | +++++     | +    |           |
| 12               | Orange-red     | ++++++ | +++++     | +    |           |
| 13               | Rich red       | ++++   | +++++     | -    |           |
| 14               | Rich red       | ++++   | +++++     | -    |           |

+: few; ++: medium; +++: many; ++++: numerous

© 2020 The Authors. Published by Rynnye Lyan Resources
purpureus mycelium that incubated in PDA medium at room temperature (25-30°C) for 14 days was visually observed (Table 1). In the early stages of growth, mycelium was white, then it changed to vibrant orange and finally became red. The color development of *M. purpureus* mycelium followed Danuri (2008).

At the early stage, the colony was observed having white color until the third day of incubation. On the fourth day of incubation, the color production started and continued to increase along with incubation period (Dikshit and Tallapragada, 2011). However, this rapidly changed into a vibrant orange (8-9 days) and later to a distinctly rich red color (14-16 days) (Manan et al., 2017; Tallapragada et al., 2017).

The strain of *M. purpureus* FNCC 6008 and JK2 produced yellow, orange, and red pigments with the red pigment being predominant. The red pigment of JK2 was stronger than that of FNCC 6008 at the final stage (14 days). The HD-CC 001 strain produced mycelium which was still white until incubation for 14 days, even though the closest part to the PDA medium appeared orange-red. The final color of 3 strains *M. purpureus* mycelium on sterilized rice at room temperature (25°C) for 14 days was visually observed that the JK2 strain gave more red pigment than FNCC 6008 and HD-CC 001 in PDA medium (Figure 1). The HD-CC 001 strain produced mycelium which was still white until incubation for 14 days, even though the closest part to the PDA medium appeared orange-red. The final color of 3 strains *M. purpureus* mycelium on sterilized rice at room temperature (25°C) for 14 days was visually observed (Table 1). In the early stages of growth, mycelium was white, then it changed to vibrant orange and finally became red. The color development of *M. purpureus* mycelium followed Danuri (2008).

At the early stage, the colony was observed having white color until the third day of incubation. On the fourth day of incubation, the color production started and continued to increase along with incubation period (Dikshit and Tallapragada, 2011). However, this rapidly changed into a vibrant orange (8-9 days) and later to a distinctly rich red color (14-16 days) (Manan et al., 2017; Tallapragada et al., 2017).

The strain of *M. purpureus* FNCC 6008 and JK2 produced yellow, orange, and red pigments with the red pigment being predominant. The red pigment of JK2 was stronger than that of FNCC 6008 at the final stage (14 days). The HD-CC 001 strain produced mycelium which was still white until incubation for 14 days, even though the closest part to the PDA medium appeared orange-red. The final color of 3 strains *M. purpureus* mycelium on sterilized rice at room temperature (25°C) for 14 days was visually observed that the JK2 strain gave more red pigment than FNCC 6008 and HD-CC 001 in PDA medium (Figure 1). The HD-CC 001 strain produced mycelium which was still white until incubation for 14 days, even though the closest part to the PDA medium appeared orange-red. The final color of 3 strains *M. purpureus* mycelium on sterilized rice at room temperature (25°C) for 14 days was visually observed (Table 1). In the early stages of growth, mycelium was white, then it changed to vibrant orange and finally became red. The color development of *M. purpureus* mycelium followed Danuri (2008).

The differences in the color of angkak indicated the differences in pigment synthesis during fermentation (Table 2). Significant differences were found in lightness (L*), yellowness (b*), and redness (a*) of angkak. Angkak fermented by FNCC 6008 possessed higher lightness (L*) and yellowness (b*), whereas angkak fermented by JK2 had higher redness (a*). The high value of lightness and yellowness, as well as the low redness, resulted in a pale red of angkak. In contrast, the low value of lightness and yellowness, as well as the high redness, showed an intense red of angkak (Ristiarini et al., 2017b). Angkak fermented by JK2 was significantly red (24.05±0.028 vs. 15.14±0.104, P = 0.000).

Table 2. Color value L*, a*, b* of angkak fermented by *M. purpureus* strain FNCC 6008 and JK2 at room temperature (25°C) for 14 days incubation

| Variable | FNCC 6008 | JK2 | P-value |
|----------|-----------|-----|---------|
| L*       | 71.54±0.534 | 56.74±0.284 | 0       |
| a*       | 15.14±0.104 | 24.05±0.028 | 0       |
| b*       | 19.25±0.182 | 17.91±0.123 | 0       |

The difference in the color of angkak produced by the strains of FNCC 6008 and JK2 was associated with the differences in the ability to utilize glucose from glycolysis. Therefore, it affected the availability of acetyl-CoA, which could have an impact on the formation of secondary metabolites, including pigment production. On the eight days of fermentation, it was clear that the expression of acetyl-CoA carboxylase converts acetyl-CoA into malonyl-CoA, as a precursor of pigment synthesis. Increased formation of malonyl-CoA and less use of malonyl-CoA for fatty acid biosynthesis can provide more malonyl-CoA precursors for pigment biosynthesis (Yang et al., 2015).

Based on the previous reports, *M. purpureus* pigment biosynthesis involves two synthesis pathways, namely the polyketide pathway and the fatty acid pathway.
Pigment formation starts from the formation of chromophore hexaketide derived from 1 mole of acetate with 5 moles of malonate by the enzyme polyketide synthase through the polyketide pathway. Simultaneously, through the biosynthetic pathway of fatty acids, the medium-chain fatty acid, such as octanoic acid forms β-keto acids. Beta-keto acids bind to the chromophore structure through trans-esterification reactions to form monascorubrin orange pigments (or rubropunctatin by binding with hexanoic acid). The reduction of monascorubrin orange pigment forms ankaflavin yellow pigment (or monascin from rubropunctatin). The amination reaction of the orange pigment (monascorubrin) with NH₃ derived from glutamic acid forms a red color (monascorubramine and rubropunctamine) (Hajjaj et al., 2000; Chen et al., 2015).

3.3 Monacolins and Citrinin Detection by LC-MS/MS

Detection of monacolin K and citrinin content in angkak powder fermented by FNCC 6008 and JK2 was carried out using LC-MS/MS. The monacolin K (m/z 405) and citrinin (m/z 251) in angkak were identified by comparing the chromatograms obtained from both samples with the chromatograms from the standard monacolin K and citrinin (Figure 3). Using the LC-MS/MS, various compounds such as dehydromonacolin K (m/z 387), mevastatin (compactin m/z 391), monacolin J (m/z 321, qualitative) (Avula et al., 2014) were also detected in both angkak powder samples. The number of various compounds detected by LC-MS/MS was shown in Table 3. Detection of several compounds with a single run at the same time requires a smaller number of samples and solvents, shorter time, and more sensitive high resolution (Monar et al., 2013). LC-MS/MS method obtains a high concentration of analytes (Song et al., 2012), informative detection (Ajdari et al., 2011) and provides very high sensitivity and accuracy (Vuković et al., 2019). The detection of specificity was determined by mass spectrometry (Avula et al., 2014; Di Dona et al., 2018).

As shown in Table 3, the angkak powder produced with FNCC 6008 and JK2 had a low content of monacolin K, as the analysis using LC-MS/MS showed that the monacolin K from both samples were under the limit of quantification (<LOQ). This indicated that the ability of both strains in synthesizing monacolin K was low. Monacolin K level of various M. purpureus strain in rice medium that analyzed by LC-MS/MS was vary in amount, NTU 601 produces 530±32 (µg/g), BCRC 31499 produces 119±21 (µg/g) (Lee et al., 2006), while angkak using FTC5391 did not contain monacolin K (Adjari et al., 2011).

The monacolin K was converted into dehydromonacolin K via dehydration, during storage. It eliminates the therapeutic effects of monacolin K (Jirasatid et al., 2013). The amount of dehydromonacolin K in both angkak was not significantly different. JK2 strain was able to produce higher mevastatin than FNCC 6008. Considering its structure and biosynthesis, the mevastatin or ML-236A or known as compactin, is similar to lovastatin (monacolin K). It also acts as an inhibitor of HMG-CoA and is highly effective in lowering plasma cholesterol levels in animals and man.

Analysis of the citrinin concentration in angkak powder showed that the strain JK2 produced lower concentration of citrinin (1.10±0.021 µg/g) compared to the strain FNCC 6008 (3.01±0.072 µg/g). The citrinin concentration in both samples was significantly different. Lee et al. (2007) reported that M. purpureus NTU 568 produce citrinin in angkak 1.89 ppm, NTU 601 0.46 ppm and NTU 301 0.37 ppm, while strain TISTR3541
produce citrinin 0.26 ppm (Adjari et al., 2011), it was detected by LC-MS/MS. Ristiarini et al. (2017b) reported the citrinin content of the commercial angkak from several region in Indonesia ranged from 17.94 ppm to 142.74 ppm.

The citrinin concentration in angkak fermented by *M. purpureus* JK2 strain was under the maximum level of citrinin allowed in foods based on the fermented rice with *M. purpureus* according to EU 212/2014 (2000 μg/kg or 2μg/g). Although Lee et al. (2010) reported that the concentration of 200 ppm citrinin in a *Monascus* fermentation product did not affect liver and kidney function as well as cause nephrotoxicity and hepatotoxicity in the animal test using rat, citrinin concentration of 2 ppm (2μg/g) was considered as a safe concentration. Therefore, the JK2 strain was considered as safe to be chosen and applied to food production.

4. Conclusion

The simultaneous detection of monacolin K and citrinin on angkak using LC-MS/MS was able to be carried out quickly. In addition, LC-MS/MS could be used to detect other monacolins. The *M. purpureus* strain JK2 produced citrinin lower than FNCC 6008, so it was preferable considering the safety aspect. However, the ability of both strains to produce monacolin K was still very low.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The research work was funded by Indonesia Endowment Fund for Education, The Indonesian Ministry of Finance (BUDI-DN LPDP). The authors are very grateful to Dr Susana Ristiarini (Widyaman Mandala University, Surabaya, Indonesia), Marlia Singgih, PhD (Microbiology Laboratory, School of Pharmacy, Bandung Institute of Technology, Indonesia) and Dr Anna Yuliana (Department of Pharmacy, STKes Bakti Tunas Husada Tasikmalaya, West Java, Indonesia) for providing *Monascus* strains, as well as Fathyah Hanum Pamungkaniingtyas, MSc. for helpful preparing manuscript.

References

Adjari, Z., Ebrahimpour, A., Abdul Manan, M., Hamid, M., Mohamad, R. and Ariff, A.B. (2011). Assessment of monacolin in the fermented products using *Monascus purpureus* FTC5391. Journal of Biomedicine and Biotechnology, 2011, 426168. https://doi.org/10.1155/2011/426168

Avula, B., Cohen, P.A., Wang, Y.H., Sagi, S., Feng, W., Wang, M., Zweigenbaum, J., Shuangcheng, M. and Khan, I.A. (2014). Chemical profiling and quantification of monacolins and citrinin in red yeast rice commercial raw materials and dietary supplements using liquid chromatography-accurate QToF mass spectrometry: Chemometrics application. Journal of Pharmaceutical and Biomedical Analysis, 100, 243–253. https://doi.org/10.1016/j.jpba.2014.07.039

Campbell, C.D. and Vederas, J.C. (2010). Biosynthesis of lovastatin and related metabolites formed by biosynthesis of lovastatin and related metabolites formed by fungal iterative PKS enzymes. Biopolymers, 93(9), 755-763. https://doi.org/10.1002/bip.21428

Chen, W., He, Y., Zhou, Y., Shao, Y., Feng, Y., Li, M. and Chen, F. (2015). Edible filamentous fungi from the species *Monascus*: early traditional fermentations, modern molecular biology, and future genomics. Comprehensive Reviews in Food Science and Food Safety, 14(5), 555-567. https://doi.org/10.1111/1541-4337.12145

Chung, C.C., Huang, T.C. and Chen, H.H. (2009). The optimization of *Monascus* fermentation process for pigments increment and citrinin reduction, presented at 2009 Ninth IEEE International Conference on Bioinformatics and Bioengineering, Taichung City, 2009. New York, NY: IEEE. https://doi.org/10.1109/BIBE.2009.33

Commission Regulation (EU) No 212/2014. (2014). 6 March 2014 amending Regulation (EC) No 1881/2006 as regards maximum levels of the contaminant citrinin in food supplements based on rice fermented with red yeast *Monascus purpureus*. Official Journal of the European Union. Retrieved on 26 June, 2020 from http://extwprlegs1.fao.org/docs/pdf/eur131703.pdf

Danuri, H. (2008). Optimizing angkak pigments and lovastatin production by *Monascus purpureus*. Hayati Journal of Biosciences, 15(2), 61-66. https://doi.org/10.4308/hjb.15.2.61

Devi, C.A. and Meera, J. (2015). Identification, mass production and application of pigment in food industry isolated from *Monascus* sp. International Journal of Bio-Technology and Research, 5(4), 1-10.

Di Donna, L., Bartella, L., Napoli, A., Sindona, G. and Mazzotti, F. (2018). Assay of lovastatin containing dietary supplement by LC-MS/MS under MRM condition. Journal of Mass Spectrometry, 53(9), 811-816. https://doi.org/10.1002/jms.4202

© 2020 The Authors. Published by Rynmye Lyen Resources
Dikshit, R. and Tallapragada, P. (2011). Monascus purpureus: A potential source for natural pigment production. Journal of Microbiology and Biotechnology Research, 1(4), 164-174.

Endo, A., Negishi, Y., Iwashita, T., Mizukawa, K. and Hirama, M. (1985). Biosynthesis of ML-236B (compactin) and monacolin K. The Journal of Antibiotics, 38(3), 444-448. https://doi.org/10.7164/antibiotics.38.444

Hajjaj, H., Klaebe, A., Goma, G., Blanc, P.J., Barbier, E. and François, J. (2000). Medium-chain fatty acids affect citrinin production in then filamentous fungus Monascus ruber. Applied and Environmental Microbiology, 66(3), 1120-1125. https://doi.org/10.1128/AEM.66.3.1120-1125.2000

Heinz, T., Schuchardt, J.P., Möller, K., Hadji, P. and Hahn, A. (2016). LDL-cholesterol-lowering effect of monacolin K from red yeast rice extract–results of a randomized, placebo-controlled intervention study. Nutrition Research, 36(10), 1162-1170. https://doi.org/10.1016/j.nutres.2016.07.005

Ji, X., Xu, J., Wang, X., Qi, P., Wei, W., Chen, X., Li, R. and Zhou, Y. (2015). Citrinin determination in red fermented rice products by optimized extraction method coupled to liquid chromatography tandem mass spectrometry (LC-MS/MS). Journal of Food Science, 80(6), T1438-T1444. https://doi.org/10.1111/1750-3841.12900

Jirasatid, S., Nopharatana, M., Kitsubun, P., Vichitsoonthongkul, T. and Tongta, A. (2013). Statistical optimization for monacolin K and yellow pigment production and citrinin reduction by Monascus purpureus in solid-state fermentation. Journal of Microbiology and Biotechnology, 23(3), 364-374. https://doi.org/10.4014/jmb.1206.06068

Lee, C.L., Wang, J.J., Kuo, S.L. and Pan, T.M. (2006). Monascus fermentation of dioscorea for increasing the production of cholesterol-lowering agent-monacolin K and antiinflammation agent-monascin. Applied Microbiology and Biotechnology, 72, 1254–1262. https://doi.org/10.1007/s00253-006-0404-8

Lee, C.L., Hung, H.K., Wang, J.J. and Pan, T.M. (2007). Improving the ratio of monacolin K to citrinin production of Monascus purpureus NTU 568 under dioscorea medium through the mediation of pH value and ethanol addition. Journal of Agricultural and Food Chemistry, 55, 6493-6502. https://doi.org/10.1021/jf0711946

Lee, C.H., Lee, C.L. and Pan, T.M. (2010). A 90-D toxicity study of Monascus-fermented products including high citrinin level. Journal of Food Science, 75(5), T91-T97. https://doi.org/10.1111/j.1750-3841.2010.01626.x

Li, Y., Zhang, F., Wang, Z.T. and Hu, Z.B. (2004). Identification and chemical profiling of monacolins in red yeast rice using high-performance liquid chromatography with photodiode array detector and mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis, 35, 1101–1112. https://doi.org/10.1016/j.jpba.2004.04.004

Manan, M.A., Mohamad, R. and Arifi, A. (2017). The morphology and structure of red pigment producing fungus: Monascus purpureus. Journal of Microbiology and Experimentation, 5(1), 00138. https://doi.org/10.15406/jmen.2017.05.00138

Mornar, A., Sertić, M. and Nigović, B. (2013). Development of a rapid LC/DAD/FLD/MS² method for the simultaneous determination of monacolins and citrinin in red fermented rice products. Journal of Agricultural and Food Chemistry, 61(5), 1072-1080. https://doi.org/10.1021/jf304881g

Nigović, B., Sertić, M. and Mornar, A. (2013). Simultaneous determination of lovastatin and citrinin in red yeast rice supplements by micellar electrokinetic capillary chromatography. Food Chemistry, 138(1),531-538. https://doi.org/10.1016/j.foodchem.2012.10.104

Ristiariini, S., Cahyanto, M.N., Widada, J. and Rahayu, E.S. (2017a). Citrinin and color analysis of angkak collected from several regions in Indonesia. Food Research,1(2), 43-49. https://doi.org/10.26656/fr.2017.2.021

Ristiariini, S., Cahyanto, M.N., Widada, J. and Rahayu, E.S. (2017b). Identification of various colored Monascus sp and its biopigments and citrinin production in RMR. International Journal of Science and Research, 6(8), 1042-1046.

Song, F., El-Demerdash, A., Lee, S.J.S.H. and Smith, R.E. (2012). Fast screening of lovastatin in red yeast rice products by flow injection tandem mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis, 57, 76–81. https://doi.org/10.1016/j.jpba.2011.08.039

Song, J., Luo, J., Ma, Z., Sun, Q., Wu, C. and Li, X. (2019). Quality and authenticity control of functional red yeast rice-A review. Molecules, 24, 1944. https://doi.org/10.3390/molecules24101944

Tallapragada, P., Dikshit, R., Phocas, M., Madhusudan, M.R. and Samprathi, A. (2017). Effect of amino acids on pigments, citrinin, and lovastatin production by Monascus purpureus under static conditions. Biologija, 63(2), 160–168. https://doi.org/10.6001/biologija.v63i2.3527

Vučković, G.L., Burić, V.P., Aleksić, G.A., Kuzmanović, S.T., Cara, M.X. and Abd, E.W.R.A.
(2017). Data acquisition of triple quadrupole LC-MS for the citrinin determination. *Matica Srpska Journal for Natural Sciences*, 2017(133), 131-141. https://doi.org/10.2298/ZMSPN1733131V

Vuković, G., Đukić, M., Bursić, V., Stojanović, T., Petrović, A., Kuzmanović, S. and Starović, M. (2019). Development and validation of LC-MS/MS method for the citrinin determination in red rice. *Journal of Agronomy, Technology and Engineering Management*, 2(1), 192-199.

Wang, T.H. and Lin, T.F. (2007). *Monascus* Rice Products. In Taylor, S. (Ed). Advances in Food and Nutrition Research, vol. 53, p. 123-159. USA: Academic Press. https://doi.org/10.1016/S1043-4526(07)53004-4

Yang, Y., Liu, B., Du, X., Li, P., Liang, B., Cheng, X., Du, L., Huang, D., Wang, L. and Wang, S. (2015). Complete genome sequence and transcriptomics analyses reveal pigment biosynthesis and regulatory mechanisms in an industrial strain, *Monascus purpureus* YY-1. *Scientific Reports*, 5(8331), 1-9. https://doi.org/10.1038/srep08331