JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

A COMBINATION OF FERMENTATION METHOD AND GERMINATED BROWN RICE (*ORYZA SATIVA*) TO ENHANCE ANTIOXIDANT ACTIVITY OF ANGKAK

*Kitisart Kraboun**

Address(es):
Division of Food Safety Management and Technology, Department of Science, Faculty of Science and Technology, Rajamangala University of Technology Krungthep, Bangkok 10120, Thailand.

*Corresponding author: Kitisart.k@mail.rmutk.ac.th

doi: 10.15414/jmbfs.2019.8.6.1290-1293

ARTICLE INFO

Received 7. 9. 2018
Revised 18. 12. 2018
Accepted 29. 1. 2019
Published 1. 6. 2019

Regular article

OPEN ACCESS

ABSTRACT

This research aimed to enhance antioxidant activities of angkak by a combination of 2-step fermentation and germinated brown rice (*Oryza sativa*) (GBR). It was showed that an appropriate combined condition of germination time of brown rice and fermentation method of angkak was 48 hours and 2-step fermentation. The highest contents of reducing sugars used by *M. purpureus* were 99.43 *%* and the highest pigment intensity, monacolin K and GABA were 3,500.89 unit /g substrate, 99.75 and 154.19 mg/kg dry weight, respectively. Whereas, the lowest IC_{50} values of DPPH and ABTS were 0.07 and 0.06 mmol Trolox equivalent /mL, respectively. However, the citrinin contents of this product were 12.37 µg/kg dry weight, indicating not exceeded following the maximum allowance level in red fermented rice.

Keywords: Enhancement; Antioxidant; Angkak; 2-step fermentation; Germinated brown rice

INTRODUCTION

Monascus pigment from angkak has been used as a coloring agent in foodstuffs, texture industries, pharmacology, medicine and cosmetics as well as used as a folk medicine to improve food digestion, blood circulation and lowering blood cholesterol levels. Angkak is also known as red koji, Hung-Chu, monascal rice, Hong Qu, ang-kak, ankak rice, red mold rice, and Beni-Koji. *Monascus* pigment is not only as a natural food coloring but also the different antioxidant potentials, i.e. its abilities donating a hydrogen atom and/or an electron, chelating redoxactive metals and inhibiting lipoxygenases decades **(Ramarathnam *et al.*, 1995; Hadjipavlou-Litina *et al.*, 2010)**. Commonly in *Monascus* fermentation, solid state fermentation (SSF) is a popular fermentation method used to produce the pigmentation and/or the antioxidant activities of angkak as well as a distinguished antioxidant, i.e. monacolin K **(Yang *et al.*, 2004; Yang *et al.*, 2006; Kongbangkerd *et al.*, 2014)**. The most important bioactive compound isolated from *Monascus* is monacolin K, which is identical to the potent cholesterol-lowering, antiatherosclerotic drug lovastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor. The enzyme produces mevalonyl-CoA, which is the important rate determining step to synthesise cholesterol, resulted in the reduction in blood pressure **(Kongbangkerd *et al.*, 2014)**.
However, a limitation of SSF affecting *Monascus* fermentation is residual reducing sugars around 2,000 $mg.kg^{-1}$ substrate in monascal products, especially glucose, after the end of the conventional *Monascus* fermentation **(Babitha *et al.*, 2007)**. Moreover, **Kongbangkerd *et al.* (2014)** reported that the reducing sugar contents were still remained at 8.00 $mg.g^{-1}$ dry weight obtained from after the conventional fermentation and were rapidly hydrolyzed until absent after 2-step fermentation.

Germinated brown rice (GBR) is called as sprouted brown rice. The process of germination increases the bio-availability of important substances by neutralizing phytic acid. The neutralizing phytic acid is able to release the proteins, vitamins, and enzymes, allowing these important nutrients to be absorbed during digestion **(Patil and Khan, 2011)**. **Choi *et al.* (2006)** reported that, beyond 24 h germination of brown rice, the enhanced contents of fructose, reducing sugars and γ -aminobutyric acid (GABA) appeared in GBR were higher 3.4 times, 2.75 times, and 7.97 times, respectively, than those appeared in the non-GBR. A study suggests that orally administered GABA increases the amount of human growth hormone (HGH) **(Powers *et al.*, 2008)**. GABA directly injected to the brain has been reported to have both stimulatory and inhibitory effects on the production of growth hormone, depending on the physiology of the individual. Certain pro-drugs of GABA (ex. picamilon) have been developed to permeate the blood–brain barrier, then separate into GABA and the carrier molecule once inside the brain. This allows for a direct increase of GABA levels throughout all areas of the brain, in a manner following the distribution pattern of the pro-drug prior to metabolism **(Powers *et al.*, 2008)**.
Therefore, a combination of 2-step fermentation and GBR, as a substrate for *Monascus purpureus*, used to produce angkak will be expected to enhance antioxidant activities and GABA contents in an angkak product.

MATERIAL AND METHODS

Microorganism

Lyophilised *Monascus purpureus* TISTR 3090 was purchased from the Thailand Institute of Scientific and Technological Research (TISTR). The strain was cultivated on Potato Dextrose Agar (PDA; Merck, Darmstadt, Germany) at 25°C for 7 days or until 10^6 spores mL^{-1} . After a pure culture was obtained, the mycelium was reinoculated into PDA slant and incubated at 25°C for 7 days or until 10^6 spores mL^{-1} before being used for angkak production.

Conventional fermentation method and 2-step fermentation of angkak

Conventional fermentation, brown rice (*Oryza sativa*) seeds were germinated at different times, i.e. 0, 12, 24, 36 and 48 hours. Then, a 100 g of germinated brown rice (GBR) was put into a flask 500 mL and was sterilized in an autoclave at 121°C for 15 min and then left until cool down. About 5 mL of 10^6 spores mL.spore $suspension^{-1}$ of *M. purpureus* obtained from actively growing slants in sterile water was inoculated into sterilized GBR and incubated at 25°C for 12 days. In conventional fermentation, it indicated that *Monascus* was appeared in the dead phase of growth curve; thus, *Monascus* was reinoculated again in step 2 fermentation in order to ferment the substrate continuously. Fermentation of step-2, angkak produced from GBR with a period time for 48 hours (obtained from the conventional fermentation) was then reinoculated with the same volume and spore suspension contents and continuously fermented with the same condition as the conventional method for another 12 days **(Kraboun *et al.*, 2013).** Then, the product was dried in an oven at 40°C for 24 h. A fine powder (20 mesh) was obtained using a mill (Retsch ultracentrifugal mill and sieving machine, Haan, Germany) (**Kongbangkerd *et al.*, 2014)**. The sample was determined Trolox

equivalent antioxidant capacity (TEAC), DPPH free radical scavenging ability, reducing sugars, monacolin K, GABA and citrinin contents.

Sample extraction for antioxidant activity assay

The extraction method described by **Yang *et al*. (2006)** was used with some modifications. A 10 g sample was extracted in a shaker with 100 mL of methanol at 170 rpm for 24 h, and the solution was filtered through Whatman no. 4 filter paper. The residue was then extracted with two additional 100-mL portions of methanol as described above. The combined methanolic extracts were then evaporated at 40°C under vacuum condition to dryness. The dried product was used for analysis of antioxidant activities.

Reducing sugars analysis

The analysis of reducing sugars contents was evaluated spectrophotometrically by a slightly modified method of Re et al. (1999). The reducing sugars released in hydrolysis were analyzed using the DNS assay **(Doner and Irwin, 1992)**. It contained a 1:1:1:1 volumetric mixture of 3,5-dinitrosalicylic acid 1%, Rochelle salt 40%, phenol 0.2%, potassium disulphide 0.5%, all in sodium hydroxide 1.5%. Typically, to 100 µL sample mixture 100 µL DNS reagent were added. The mixture was incubated in a boiling water bath for 5 min. After cooling to room temperature, the absorbance of the supernatant at 540 nm was measured.

Trolox equivalent antioxidant capacity (TEAC)

For ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay, antioxidant activity of angkak extracts against $ABTS^+$ radical was evaluated spectrophotometrically by a slightly modified method of Re et al. (1999). The TEAC assay is based on the scavenging of $ABTS^+$ radical converting into a colourless product. The degree of decolourisation induced by a compound is related to that induced by Trolox, giving the 'TEAC value'. The $ABTS^+$ radical was produced by the reaction between 2 mL of 7 mM ABTS solution and 40 µL of 2.45 mM potassium persulphate solution and stored in the dark at room temperature for 16 h. Before usage, the $ABTS^+$ solution was diluted to get an absorbance of 0.700±0.025 at 734 nm with ethanol. For the assay, the resulting solution was mixed with 300 µL of sample of each monascal waxy corn extract (1–20 mg/mL). The absorbance was read at 30 °C after exactly 6 min. The obtained absorbance of samples was compared with a standard curve from the corresponding readings of trolox (0.4-0.04 mM). The total antioxidant capacities (TAC) were estimated as Trolox equivalent antioxidant capacity (TEAC) by interpolation to 50% inhibition ($TEAC_{50}$).

DPPH radical scavenging activity

The scavenging activity (H/e-transferring ability) against 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was measured spectrophotometrically by following **Velazquez *et al*. (2003)**. The extract (40 µL) with varying concentrations (1-20 mg/mL) was mixed with 200 µL of 0.02 mM DPPH solution and methanol 4 mL. Samples were kept for 15 min at 25 °C and the absorbance was measured at 517 nm. The absorbance of a blank sample containing the same amount of solvent was also measured. The extent of decolourisation is calculated as a percentage reduction of absorbance, and this is determined as a function of concentration and calculated relative to the 0.1-0.01 mM of equivalent Trolox concentration. The radical scavenging activity is expressed in mmol of equivalent trolox per gram of sample (mmol Trolox equivalent /mL) with interpolation to 50% inhibition (IC_{50}).

Monacolin K analysis

An 0.5 g sample was extracted with 25 mL of 70% ethanol by using a shaker at 50°C for 2 h, followed by filtration through a 0.2 µm membrane and the extract was analysed by HPLC. The HPLC system consisted of Shimadzu LC-10AT VP Liquid Chromatograph, a FCV-10AL VP pump, an LDC Analytical SpectroMonitor 3100 detector set at 238 nm and an LDC Analytical CI-4100 integrator. A chromatography column Ascentis C18, 5µm, 250×4.6 mm was connected to a 20 µL loop injector. An isocratic mobile phase of acetonitrile:water in the ratio of 65:35 (by vol.) was used. The flow rate and temperature were 1.0 mL/min and 28°C, respectively **(Chayawat *et al*., 2009)**. Monacolin K dissolved in 70% ethanol was used as a standard.

GABA analysis

One gram of dried angkak powder was extracted with 5 ml water at 60°C for 2 h with vigorously shaking. After 12,000 x g cencentrifuging for 20 min at 4°C, 400 µl aliquot of supernatant (or standard solution of GABA) was vacuum-dried. The residue was dissolved in 50 µl ethanol-water-triethylamine (2:2:1) solution, and the mixture was then evaporated to dryness under vacuum until dry and redissolved again in 40 µl ethanol-water-triethylamine-phenylisothiocyanate solution (6:1:1:1). The final mixture was allowed to react for 20 min at room temperature to form phenylisothiocyanate-GABA (PTC-GABA).
Procedure of HPLC analysis described by **Wang *et al*. (2004)** was slightly modified. Briefly, the dry residue containing PTC-GABA was dissolved by adding 400 µl mobile phase that consisted of 80% solution A (aqueous solution of 8.205 g sodium acetate, 0.5 ml triethylamine, 0.7 ml acetic acid, and 5.0 ml acetonitrile in 1000 ml distilled water, pH 5.8) and 20% solution B (acetonitrile-water, 60:40, pH 5.8). Chromatographic separation was conducted on a Shim-pack VP-ODS C18 column (4.6 × 150 mm *i.d*., 5 µm). The eluent was pumped at a flow rate at 0.6 ml/min. Temperature of column oven was 46°C and UV detection wavelength was set at 254 nm.

Citrinin analysis

Citrinin analysis was described by **Lim *et al*. (2010)**. A 1 g sample was extracted with a solution (acetone : ethyl acetate = 1:1, v/v) at 65ºC for 90 min under vigorous shaking. The supernatant was obtained by centrifugation at 1,600 x g for 10 min followed by filtration through a 0.45 µm PTFE (Polytetrafluoroethylene) filter unit (National Scientific, Rockwood, TN). The citrinin was determined by HPLC using a chromatography column Ascentis C18 column (4.6 x 250 mm). The mobile phase consisted of methanol/acetonitrile/ 0.1% phosphoric acid (3:3:4, v:v) and the analysis was performed with a fluorescence detector set at excitation and emission wavelengths of 330 and 500 nm, respectively. The flow rate was 0.6 mL/min and the sample was spiked to confirm the presence of citrinin.

Statistical analysis

All determinations were performed in triplicate and results were expressed as the mean±standard deviation calculated using spreadsheet software Microsoft Excel. This was carried out in a completely randomized experimental design **(**CRD**)** and the data were analysed by an analysis of variance ($p \leq 0.05$) and means were compared using Duncan's new multiple range test. The results were processed by SPSS 16.0 (SPSS Inc., Chicago, IL, USA) for Windows.

RESULTS AND DISCUSSION

Reducing sugars and pigment intensity of angkak produced from GBR with different germination times and different fermentation methods

The contents of reducing sugars and pigment intensity of angkak using germinated brown rice (GBR) with different germination times as a substrate are shown in Table 1. The contents of reducing sugars of angkak obtained from the conventional fermentation decreased with increasing pigment intensity when using GBR with increased germination times. Moreover, using GBR with a germination period for 48 hours, *Monascus purpureus* could produce the highest pigment intensity and use the highest contents of reducing sugars indicating reducing sugar decreased 28.08 %. This was a cause of *M. purpureus* applying the variously significant substances such as vitamins, minerals and dietary fibers as well as the antioxidants (GABA and phenolic compounds) occurred during the seed germination process. Furthermore, reducing sugars also used as a substrate so that the significant substances were continuously produced affecting an increase of pigment intensity **(Chung *et al*., 2009)**.
Therefore, the angkak produced from GBR with a germination period for 48 hours via the conventional method was continuously fermented by *M. purpureus*. In 2-step fermentation, 99.43 % of the contents of reducing sugars were applied by *M. purpureus* and they were still residual at 0.01 mg/g substrate; whereas, the pigment intensity was increased to 3,500.89 unit /g substrate. This result indicated that 2-step fermentation led to increasingly used contents of reducing sugars to be a substrate for *M. purpureus* affecting higher pigment intensity **(Kongbangkerd *et al*., 2014)**. This was in agreement with **Kongbangkerd *et al*. (2014)** who reported that the pigment intensity of monascal waxy corn from 2-step fermentation was 3,500 unit /g substrate and the contents of reducing sugars were exhausted compared with those of monascal waxy corn from the conventional method (500 unit /g of substrate of pigment intensity and 8 mg/g of reducing sugars). In addition, the 2 kinds of hydrolyzing enzymes such as α-amylase and glucoamylase were continuously appeared during the conventional and 2-step fermentations so that this may be another cause to enhance hydrolysis of the substrates for pigment production
(Babitha *et al*., 2007).

Table 1 Contents of reducing sugars and pigment intensity of angkak produced from germinated brown rice (GBR) with different germination times and different fermentation methods

Fermentation methods	Germination times of brown rice (hour)	Reducing sugars (mg/g substrate)**,***	Decreased percentages of reducing sugar (%)*	Pigment intensity (unit /g substrate)**,***
Conventional batch	0	1.78 ± 0.00^{e}	0	200.89 ± 2.50^{a}
Conventional batch	12	1.68 ± 0.02^{d}	5.61	250.78 ± 9.00^{b}
Conventional batch	24	1.53 ± 0.00^{c}	85.95	369.85 ± 3.50^{c}
Conventional batch	36	1.33 ± 0.05^{b}	14.04	459.79 ± 5.70^{d}
Conventional batch	48	1.28 ± 0.33^{b}	28.08	500.98 ± 4.90^{e}
2-step fermentation	-	0.01 ± 0.00^{a}	99.43	$3{,}500.89 \pm 15.34^{f}$

*Decreased percentages of reducing sugars are the used contents compared with the contents of reducing sugars angkak using GBR with a germination period for 0 hour.
**Different letters within the same column indicate statistical differences (one-way ANOVA and Duncan test, $p \leq 0.05$).
***Values are mean ± S.D of triplicate determinations.

IC_{50} of DPPH and ABTS of angkak produced from GBR with different germination times and different fermentation methods

The IC_{50} values of DPPH and ABTS of angkak produced from GBR with different germination times and different fermentation methods are shown in Figure 1. It was found that, in the conventional method, the IC_{50} values of DPPH and ABTS of angkak were significantly lower when using GBR with increased germination times ($p \leq 0.05$). The pigment of angkak using GBR with 48 hours of germination time was lower IC_{50} values of DPPH and ABTS than that from GBR with 0 hour of germination time (without germination), which had the lowest IC_{50} values of DPPH and ABTS showing 0.15 and 0.17 mmol Trolox equivalent /mL, respectively. In 2-step fermentation, the IC_{50} values of ABTS and DPPH of pigment from angkak were 0.07 and 0.06 mmol Trolox equivalent /mL, respectively, which were 2 times lower than those from angkak using GBR with a germination period for 48 hours through the conventional method. It seemed that the pigment extracted from angkak produced from a combination of GBR and 2-step fermentation might have the presence of the glycone part, which masked hydrogen donation property of the pigment, indicating an important feature for free radical scavenging **(Bhanja *et al.*, 2008)**. Moreover, monacolin K and/or total phenols occurred in the pigment from angkak affected the inhibition of the formation of ABTS• by one-electron oxidants, showing an effectiveness as an electron donor **(Hagerman *et al.*, 1998; Yang *et al.*, 2006)**.

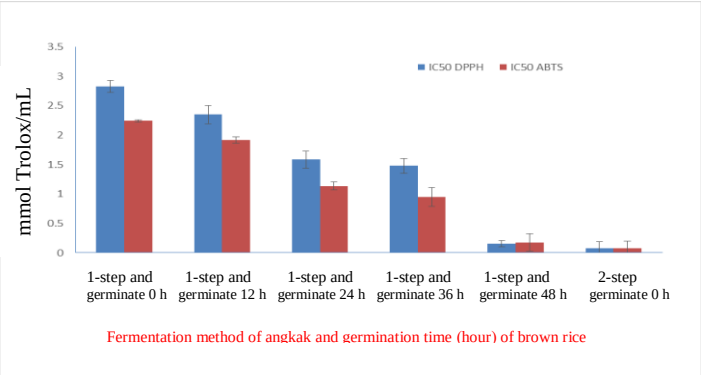

Figure 1 IC_{50} values of DPPH and ABTS of angkak produced from germinated brown rice (GBR) with different germination times and different fermentation methods.This was carried out in a completely randomized experimental design (CRD) and the data were analysed by an analysis of variance ($p \leq 0.05$) and means were were compared using Duncan's new multiple range test.

Monacolin K, GABA and citrinin of angkak produced from GBR with different germination times and different fermentation methods

The contents of monacolin K, GABA and citrinin of angkak produced from GBR with different germination times and different fermentation methods are shown in Figure 2. As compared to the angkak products using GBR with different germination times and the conventional method, the contents of monacolin K, GABA and citrinin were significantly higher when using GBR with increased germination times ($p \leq 0.05$). In angkak obtained from GBR with a germination period for 48 hours, the highest contents of monacolin K, GABA and citrinin were 77.16 and 120.59 mg/kg dry weight and 10.17 µg/kg dry weight, respectively. The contents of monacolin K (99.75 mg/kg dry weight), GABA (154.19 mg/kg dry weight) and citrinin (12.37 µg/kg dry weight) of angkak via 2-step fermentation were higher than those of GBR with a germination period for 48 hours through the conventional method. The monacolin K, GABA and citrinin contents of angkak via 2-step fermentation were in agreement with those reported for monascal waxy corn **(Kongbangkerd *et al.*, 2014)**. In this experiment, the fermentation temperature was 25ºC which was appropriate for monacolin K and GABA **(Tsukahara *et al.*, 2009)**. **Su *et al.* (2003)** confirmed that solid state fermentation (SSF) was a type of fermentation that could lead to not only the yields of the products but also a low energy requirement, which reduced the production costs. In addition, this experiment using SSF which was conducted by incubation at 25ºC indicating the highest yield of monacolin K. Furthermore, **Pengnoi *et al.* (2017)** comfirmed that a temperature of 25°C was appropriate for the angkak production due to increased to 93.07% of monacolin K content. However, angkak is frequently contaminated with citrinin. Contamination with citrinin is a problem influencing acceptability because it is a mycotoxin which damages the liver and kidneys of mammals **(Kongbangkerd *et al.*, 2014)**. Although the contents of citrinin of angkak from 2-step fermentation were 12.37 µg/kg dry weight, the citrinin contents were not exceeded following the maximum allowance level in red fermented rice. According to the legislation of many countries, Japan has issued an advisory limit of 200 µg kg^{-1} of citrinin in commercially agricultural products. The limit set by the Chinese Food and Drug Administration is 20 µg kg^{-1} of citrinin, while the European Union has recommended a citrinin limit of 100 µg kg^{-1} **(Shi and Pan, 2011)**. This study result was a success using a selected condition, which produced the low contents of citrinin and the high contents of monacolin K and GABA to be potential for providing safe functional food.

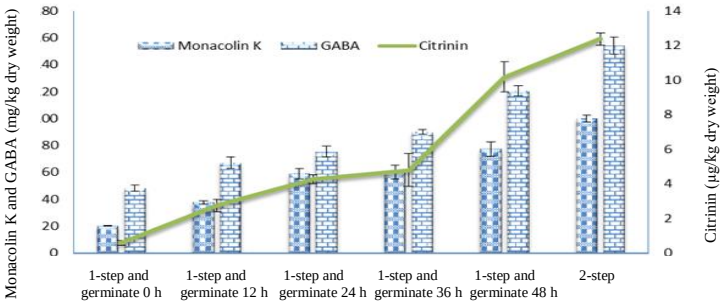

Figure 2 Monacolin K, GABA and citrinin of angkak produced from germinated brown rice (GBR) with different germination times and different fermentation methods. This was carried out in a completely randomized experimental design (CRD) and the data were analysed by an analysis of variance ($p \leq 0.05$) and means were compared using Duncan's new multiple range test.

CONCLUSION

The increasing time of germination of GBR impacted on reducing sugars usage by *Monascus purpureus*. Hence, the used contents of reducing sugars of angkak obtained from a combination of GBR with a germination period for 48 hours and 2-step fermentation was 99.43 %; therefore, the highest contents of monacolin K and GABA were produced as well as the lowest IC_{50} values of DPPH and ABTS. Moreover, the citrinin contents were not exceeded following the maximum allowance level in the red fermented rice product as well.

Acknowledgements: This work was funded by Rajamangala University of Technology Krungthep. Moreover, The author would like to thank all staffs of the faculty for maintenance and operation of the scientific laboratory and equipment.

REFERENCES

Babitha S., Soccol R.C. & Pandey A. (2007). Solid-state fermentation for the production of monascus pigments from jackfruit seed. *Bioresource Technology*, 98: 1554-1560.
Bhanja T., Rout S., Banerjee R. & Bhattacharyya B.C. (2008). Studies on the performance of a new bioreactor for improving antioxidant potential of rice.

LWT-Food Science and Technology. 41: 1459–1465. http://dx.doi.org/10.1016/j.lwt.2007.08.015

Chayawat J., Jareonkitmongkol S., Songsasen A. & Yongsmith B. (2009). Pigments and anti-cholesterol agent production by *Monascus kaoliang* KB 9 and its color mutants in rice solid cultures. *Kasetsart Journal*, 43: 696-702.

Choi I., Kim D., Son J., Yang C., Chun J. & Kim K. (2006) Physicochemical properties of giant embryo brown rice (Keunnunbyeo). *Agricultural chemistry and Biotechnology*, 49: 95-100.

Chung H.J., Jang S.H., Cho H. Y. & Lim S.T. (2009). Effects of steeping and anaerobic treatment on GABA (γ-aminobutyric acid) content in germinated waxy hull-less barley. *LWT-Food Science and Technology*, 42; 1712-1716. https://doi.org/10.1016/j.lwt.2009.04.007

Doner L.W. & Irwin P.L. (1992)**.** Assay of reducing end-groups in oligosaccharide homologues with 2,2'- bicinchoninate**.** *Analytical Biochemistry*, 202, 1: 50-53. https://doi.org/10.1016/0003-2697(92)90204-K

Hadjipavlou-Litina D., Magoulas G.E., Krokidis M. & Papaioannou D. (2010). Syntheses and evaluation of the antioxidant activity of acitretin analogs with amide bond(s) in the polyene spacer. *European Journal of Medicinal Chemistry*, 45: 298-310. https://doi.org/10.1016/j.ejmech.2009.10.012

Hagerman A.E., Riedl K.M., Jones G.A., Sovik K.N., Ritchard N.T., Hartzfeld, P.W. & Riechel T.L. **(**1998**)**. High molecular weight plant polyphenolics (Tannins) as biological antioxidants. *Journal of Agricultural and Food Chemistry*, 46: 1887-1892. http://doi.org/10.1021/jf970975b

Kongbangkerd T., Tochampa W., Chatdamrong W. & Kraboun K. (2014), Enhancement of antioxidant activity of monascal waxy corn by a 2-step fermentation. *International Journal of Food Science and Technology*, 49: 1707–1714. https://doi.org/10.1111/ijfs.12479

Lim J.Y., Kim J.J., Lee D.S., Kim G.H., Shim J.Y., Lee I. & Imm J.Y. (2010). Physicochemical characteristics and production of whole soymilk from *Monascus* fermented soybeans. *Food Chemistry*, 120: 255-260. http://dx.doi.org/10.1016/j.foodchem.2009.10.017

Patil S.B. & Khan K. **(**2011**).** Germinated brown rice as a value added rice product: A review**.** *Journal of Food Science and Technology*, 48: 661–667. http://doi.org/10.1007/s13197-011-0232-4

Pengnoi P., Mahawan R., Khanongnuch C., Lumyong S. (2017). Antioxidant properties and production of monacolin k, citrinin, and red pigments during solid state fermentation of purple rice (*Oryzae sativa*) varieties by *Monascus purpureus*. *Czech Journal of Food Sciences*, 35: 32-39. https://doi.org/10.17221/154/2016-CJFS

Ramarathnam N., Osawa T., Ochi H. & Kawakishi, S. (1995). The contribution of plant food antioxidants to human health. *Trends in Food Science and Technology*, 6: 75-82.

Powers M.E., Yarrow J.F., McCoy S.C. & Borst S.E. **(**2008). Growth hormone isoform responses to GABA ingestion at rest and after exercise. *Medicine and Science in Sports and Exercise*, 40: 104-110. https://doi.org/10.1249/mss.0b013e318158b518

Re R., Pellegrini N., Proteggente A., Pannala A., Yanh M. & Rice-Evans C. (1999). Antioxidant activity applying an improved ABTS radical cation decolourization assay. Free Radical Biology and Medicine, 26: 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Shi Y.C. & Pan T.M. (2011). Beneficial effects of *Monascus purpureus* NTU 568-fermented products: a review. *Applied Microbiologyand Biotechnology*, 90: 1207-1217. http://dx.doi.org/10.1007/s00253-011-3202-x

Su Y.C., Wang J.J., Lin T.T. & Pan T.M. (2003). Production of the secondary metabolites γ-aminobutyric acid and monacolin K by *Monascus*. *Journal of Industrial Microbiology Biotechnology*, 30: 41-46. http://doi.org/10.1007/s10295-002-0001-5

Tsukahara M., Shinzato N., Tamaki Y., Namihira T. & Matsui T. (2009). Red yeast rice fermentation by selected *Monascus* sp. with deep-red color, lovastatin production but no citrinin, and effect of temperature-shift cultivation on lovastatin production. *Applied Biochemistry and Biotechnology*, 158: 476-482. http://dx.doi.org/10.1007/s12010-009-8553-8

Velazquez E., Tournier H.A., Mordujovich de Buschiazzo P., Saavedra G. & Schinella G.R. (2003). Antioxidant activity of Paraguayan plant extracts. *Fitoterapia*, 74: 91-97. http://dx.doi.org/10.1016/S0367-326X(02)00293-9

Wang J.J, Lee C.L & Pan Z.M. (2004). Modified mutation method for screening low citrinin-producing strains of Monascus purpureus on rice culture. *Journal of Agriculture and Food Chemistry*, 52: 6977-6982. http://doi.org/10.1021/jf049783o

Yang J.H., Tseng Y.H., Chang H.L., Lee Y.L. & Mau J.L. (2004). Storage stability of monascal adlay. *Food Chemistry*, 90: 303-309. https://doi.org/10.1016/j.foodchem.2004.03.053

Yang J.H., Tseng Y.H., Lee Y.L., & Mau J.L. (2006). Antioxidant properties of methanolic extracts from monascal rice. *LWT-Food Science and Technology*, 39: 740-747. http://dx.doi.org/10.1016/j.lwt.2005.06.002