Biocatalysis and Agricultural Biotechnology 18 (2019) 101101

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Maltose syrup residue as the substrate for *Monascus* pigments production

Camila Fernanda Dias de Oliveira, Jaquelinne Pires Vital da Costa, Francielo Vendruscolo*

College of Agronomy, Federal University of Goiás, Goiânia, GO, 74.690-900, Brazil

ARTICLE INFO

Keywords:
Radial growth rate
Submerged fermentation
Thermal stability
Microbial pigments
Agroindustrial residue

ABSTRACT

The objective of this study was to determine the effect of pH on the growth rate and production of pigments by *Monascus ruber* from maltose syrup residue as a substrate. The cultivations were carried out in solid and submerged media under different pH conditions. The highest radial growth rate occurred when the *Monascus ruber* was cultivated at pH 6.5 and the production of yellow pigments occurred at low pH values (2.0 and 2.5), whereas the production of orange pigments occurred at pH 3.0 and 3.5 and that of red pigments when the fungus was cultivated above pH 4.0. The results showed that the pH value had a significant influence on the radial growth rate and production of pigments, and especially on the profile of yellow, orange and red pigments when cultivated in a medium containing maltose syrup. Maltose syrup was shown to be a potential substrate for the production of pigments by *Monascus ruber*, potentiating the use of byproducts from the syrup processing industry to produce microbial pigments.

1. Introduction

Colors are added to foods to restore the original appearance affected during the processing, storage, packaging and distribution steps. Thus natural pigments are widely employed in foods due to their great importance in increasing product acceptance, substituting potentially prejudicial synthetic pigments (Hilares et al., 2018; Lv et al., 2017).

One alternative way of producing natural food dyes is the application of biotechnological tools. Different species of bacteria, fungi, yeasts and algae can produce pigments such as carotenoids (Chacón-Ordoñez et al., 2017), melanins, flavins, quinones (Dufossé et al., 2005; Marioa et al., 2016), and, more specifically, monascines (Bühler et al., 2013; Lee et al., 2016), amongst the molecules produced. In addition the secondary metabolites produced by some fungi are extremely important and present an economic impact, such as the natural pigments produced by the *Monascus purpureus* and *Monascus ruber*, *Blakeslea trispora* (Wana et al., 2016; Wanga et al., 2017), *Penicillium* sp. (Domínguez-Santosa et al., 2017; Long et al., 2017) and *Aspergillus* sp. (Dasan et al., 2017).

Monascus species are traditionally used to produce food dyes, fermented foods, alcoholic beverages, rice, frankfurters, meat and vegetables in countries such as China, Taiwan, Thailand, Japan, Indonesia and The Philippines (Ning et al., 2017). Apart from the pigments, secondary metabolites such as monacolin K, citrinin and antioxidants are also produced (Wu, 2015). Monacolin K revolutionized the treatment of cholesterol, since it is a competitive inhibitor of HMG-CoA reductase (3-hydroxy-3-methylglutaryl-coenzyme A) the determinant enzyme in cholesterol synthesis (Zhang et al., 2018).

Monascus produces six main pigments which can be divided into orange, yellow and red pigments (Yuliana et al., 2017). The formation of pigments occurs as from the condensation of 1 mol of acetate with 5 mol of malonate, leading to the production of the chromophore hexacetide in the cytosol via the multi-enzyme complex polyketide synthase. The medium chain fatty acids, such as, for example, octanoic acid, are synthesized via the fatty acid metabolic route and bind to the chromophore structure by way of a transesterification reaction, producing the orange pigment (monascorubrin - $C_{23}H_{26}O_5$ or rubropunctatin - $C_{21}H_{22}O_5$ in transesterification with octanoic acid). The reduction of the orange pigment monascorubrin gives rise to the yellow pigment (ankaflavin - $C_{23}H_{30}O_5$ or monascin - $C_{21}H_{26}O_5$ to rubropunctatin). The red pigments (monascorubramine - $C_{23}H_{27}NO_4$ and rubropunctamine - $C_{21}H_{23}NO_4$) are produced by the reaction of the orange pigment with compounds containing NH_2 (Aboyibor et a., 2018).

The production of *Monascus* pigments is related to various factors, such as the type of fermentation, solid or submerged, the microbial species, the pH value, the nitrogen sources, the carbon source and nutrients, temperature, dissolved oxygen and aeration (Zhang et al., 2015; Kang et al., 2013). Of these factors, the pH has been defined as the potential that influences the production of pigments by *Monascus* sp., pH values close to neutrality leading to the formation of red pigments or red-derived pigments. It has also been reported that the use of a rice medium with the pH controlled at 3.0 results in the production of

* Corresponding author.
E-mail address: francielo160679@ufg.br (F. Vendruscolo).

https://doi.org/10.1016/j.bcab.2019.101101
Received 22 January 2019; Received in revised form 4 February 2019; Accepted 17 March 2019
Available online 19 March 2019
1878-8181/

yellow pigments, and a rice medium with neutral pH in the formation of red pigments (Shi et al., 2015). In addition the substrate also stands out in microbial development, the ideal substrate being that which provides all the nutrients necessary for the optimal functioning of the fungi (Xu et al., 2006).

The use of agro-industrial residues from the food industry could be a great opportunity to reduce the costs of these pigments. Quantitatively maltose syrup is the main byproduct of the grain malting process particularly that of barley, and commercially by starch hydrolysis, catalyzed by specific enzymes, using β-amylase from *Bacillus* species, although the β-amylase from barley, soybean or sweet potato seeds can also be used (Cereda and Vilpoux, 2003). When discarded in the environment they constitute a problem, since they contain elevated carbohydrate contents, resulting in a high chemical oxygen demand (Cordeiro et al., 2012). Thus the present study aimed to re-use this agro-industrial residue to manufacture the natural pigments produced by *Monascus ruber* CCT 3802 in solid and submerged cultures under different pH conditions.

2. Material and methods

2.1. Microorganism

Monascus ruber CCT 3802 was obtained from the André Tosello Culture Collection (Campinas, SP, Brazil) and maintained in malt extract agar (MEA) (50 g L^{-1}) (Nuclear, São Paulo, Brazil). Agar slants in test tubes and Roux bottles sterilized at 121 °C for 15 min were inoculated, incubated at 30 °C for 7 days and then maintained at 4 °C.

2.2. Cultivation in a solid medium

The solid culture medium was obtained by mixing (MEA) (50 g L^{-1}) with 5 g L^{-1} maltose syrup (containing 70.0 g L^{-1} maltose and 2.23 g L^{-1} glucose) donated by the company *Instituto de Fosfatos Biológicos* (Goiânia, GO, Brazil). The culture medium was autoclaved at 121 °C for 15 min, cooled, and the pH value adjusted to the range from 2 to 7 with 10% m/v HCl or NaOH using a previously calibrated Mettler Toledo pH-meter (Brasília, DF, Brazil). While still liquid, the medium was poured into 100 mm Petri dishes. After solidification, the culture media were inoculated with a spore suspension was prepared by adding three loopfuls of *Monascus ruber* to 1 mL of 0.2% (w/v) bacteriological agar, previously autoclaved at 121 °C for 15 min. The inoculations were done using a 1.4 mm diameter micropipette with a sterile tip, immersing the tip in the suspension and transferring to the solidified medium.

After inoculation, three radii were drawn on the bottom of each Petri dish passing through the inoculation point, and the plates incubated in a BOD chamber at 30 °C. Every 24 h, the diameters of the colonies were measured, and the radial growth velocity estimated from the linear regression of the evolution of the radii of the *Monascus ruber* colonies as a function of cultivation time (Equation (1)).

$$r(t) = V_{RG}.\ t + a \quad (1)$$

Where *r(t)* is the radius of the colony (mm); α is the linear regression constant, corresponding to the radius of the inoculator (1.4 mm); V_{RG} is the velocity of radial growth (mm h^{-1}) and *t* the cultivation time (h). Each experiment was repeated 5 times.

2.2.1. Pigment

At the end of the measurements of radial evolution as a function of cultivation time, the *Monascus ruber* CCT 3802 colonies, cultivated in different pH values, were scraped from the agar surface and placed in 250 mL conical flasks containing 30 mL of 95 ºGL ethyl alcohol. The flasks were incubated at 40 °C for 2 h in a thermostatically controlled water bath (Spencer, SP, Brazil) with agitation at 100 rpm. The contents were then filtered through Whatman nº 1 qualitative filter paper (Madiston, England) and the filtrate containing the pigments submitted to analytical determinations on spectrophotometry (Cirrus 80, Piracicaba, SP, Brazil) at 410 and 510 nm for yellow and red pigments, respectively; whereas the solid part was submitted to a determination of the biomass.

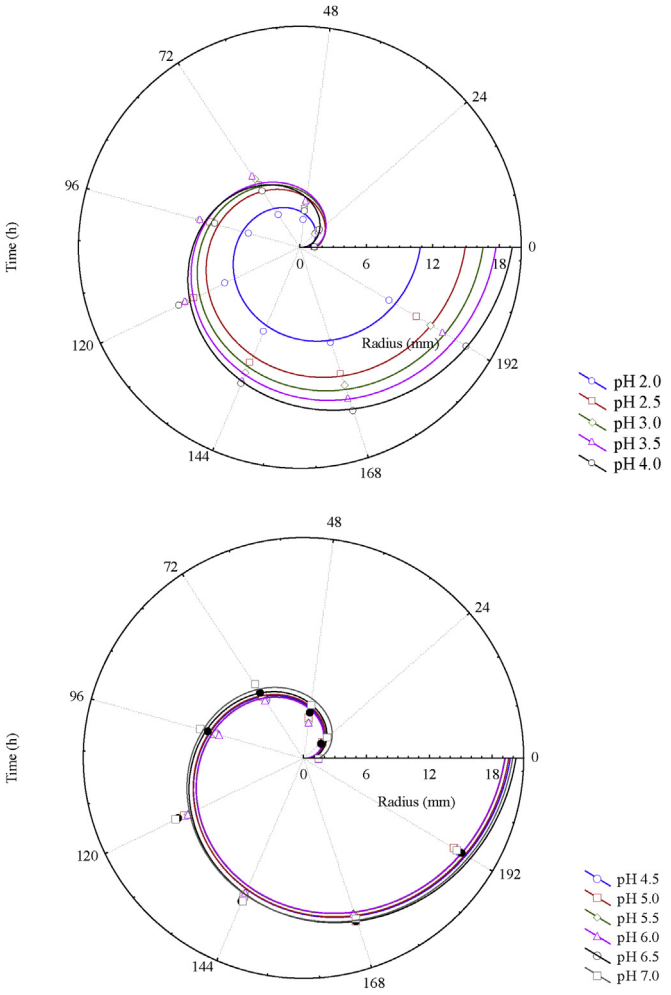

Fig. 1. Evolution of the radii of the *Monascus ruber* CCT 3802 colonies cultivated under different pH conditions.

Table 1
Radial growth velocity, equation for the radius of the *Monascus ruber* CCT 3802 colonies as a function of culture time, determination coefficient R^2 and visual aspect of the colonies cultivated under different pH conditions.

pH	V_{RG} (mm h^{-1})	Regression equation	R^2	Visual aspect
2.0	0.0434 ± 0.051^{d}	$r = 0.0434t + 1.4$	0.9788	Yellow
2.5	0.0646 ± 0.002^{c}	$r = 0.0646t + 1.4$	0.9898	Yellow
3.0	0.0721 ± 0.004^{bc}	$r = 0.0721t + 1.4$	0.9799	Orange
3.5	0.0800 ± 0.002^{ab}	$r = 0.0800t + 1.4$	0.9857	Orange
4.0	0.0804 ± 0.007^{ab}	$r = 0.0804t + 1.4$	0.9842	Red
4.5	0.0808 ± 0.003^{ab}	$r = 0.0808t + 1.4$	0.9962	Red
5.0	0.0839 ± 0.003^{ab}	$r = 0.0830t + 1.4$	0.9882	Red
5.5	0.0830 ± 0.002^{ab}	$r = 0.0839t + 1.4$	0.9943	Red
6.0	0.0840 ± 0.007^{a}	$r = 0.0850t + 1.4$	0.9956	Red
6.5	0.0888 ± 0.002^{a}	$r = 0.0888t + 1.4$	0.9834	Red
7.0	0.0883 ± 0.002^{a}	$r = 0.0883t + 1.4$	0.9987	Red

V_{RG}: Radial growth velocity; *r*: colony radius (mm); *t*: time (h). Means followed by the same letter in the same column do not differ significantly according to Tukey's test at a level of significance of $p \leq 0.05$).

2.2.2. Determination of biomass

The biomass retained by the filter paper was autoclaved at 121 °C

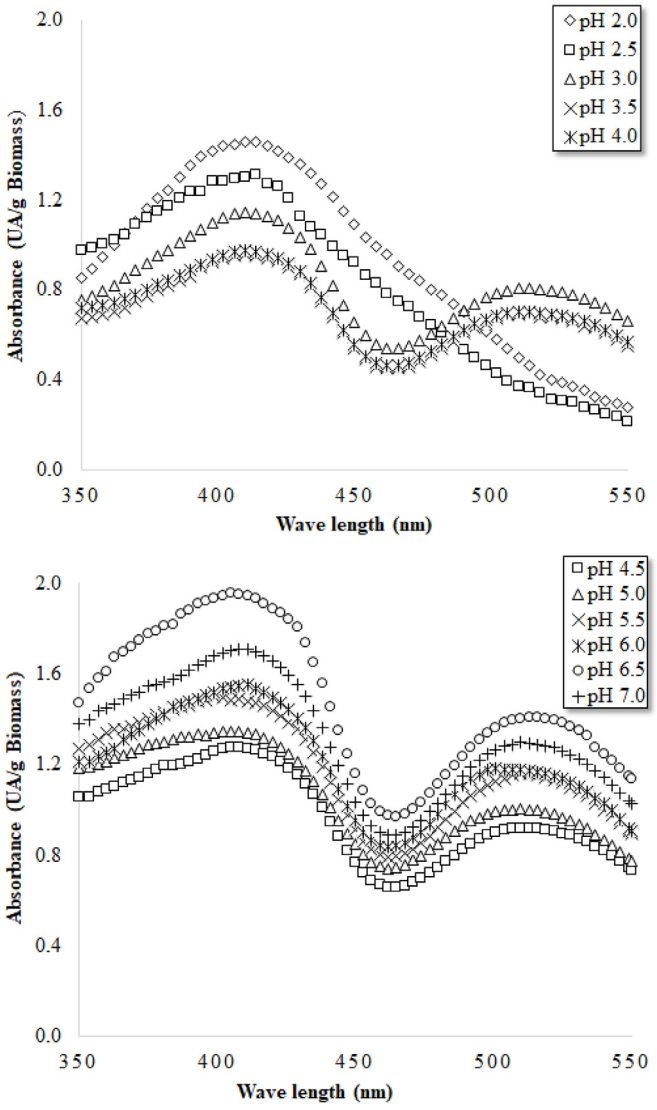

Fig. 2. Scanning spectrum of the pigments produced by *Monascus ruber* CCT 3802 cultivated under different pH conditions.

for 15 min in conical flasks containing 30 mL of distilled water, and the colonies separated, dried and weighed on an analytical balance.

2.2.3. Scanning spectrophotometry

The pigments produced by the cultures under different pH conditions were submitted to scanning spectrophotometry (Cirrus 80, Piracicaba, SP, Brazil) in the range from 350 to 550 nm (Vendruscolo et al., 2016).

2.2.4. Determination of the color parameters

The color parameters of L^* (luminosity - pure white to pure black), a^* (intensity of green (−) and red (+)) and b^* (intensity of blue (−) and yellow (+)) were determined by a direct reading of the total transmittance using a ColorQuest XE Minolta colorimeter (Brasília, DF, Brazil) according to the methodology proposed by Savadkoohi et al. (2014). The saturation index or chroma (C^*) and the angular hue angle ($h°$), were determined from equations (2) and (3), respectively:

$$C^* = \sqrt{(a^{*2} + b^{*2})} \quad (2)$$

$$h° = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (3)$$

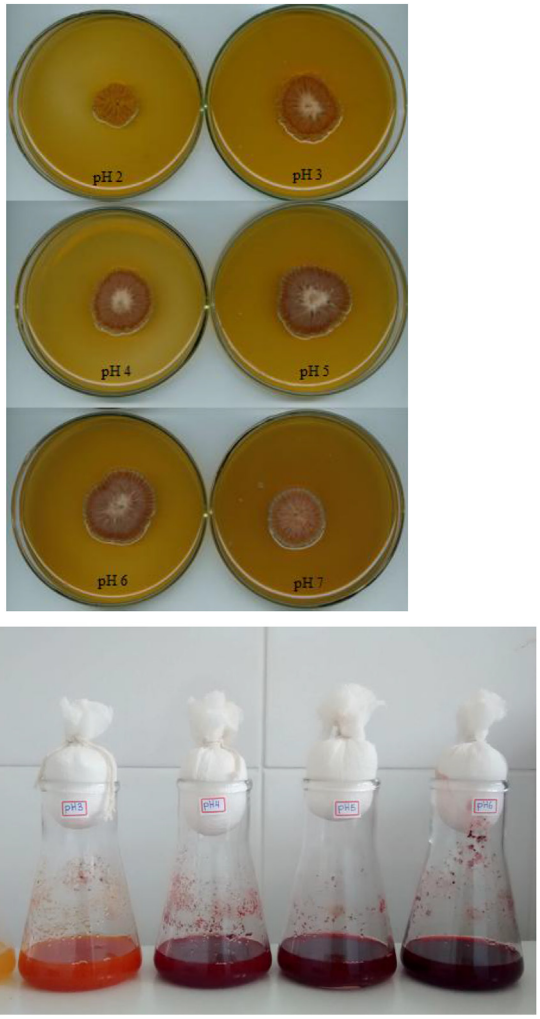

Fig. 3. Visual aspect of the *Monascus ruber* CCT 3802 cultivated under different pH conditions.

2.3. Submerged cultivations

2.3.1. Culture medium

The submerged cultures were carried out in 250 mL conical flasks containing 120 mL of a culture medium containing, per liter: 5 g glycine; 5 g K_2HPO_4; 5 g KH_2PO_4; 0.1 g $CaCl_2$; 0.5 g $MgSO_4{\cdot}7H_2O$; 0.01 g $FeSO_4{\cdot}7H_2O$; 0.01 g $ZnSO_4{\cdot}7H_2O$ and 0.03 g $MnSO_4{\cdot}H_2O$.

2.3.2. Inoculation and culturing

The previously sterilized culture medium was inoculated with 10% (v/v) of a spore solution containing approx. 4×10^6 spores mL^{-1}. The pH was adjusted to the range from 2.0 to 7.0 using a Mettler Toledo pH-meter (Brasília, DF, Brazil) using 10% NaOH and HCl (m/v) solutions (A.O.A.C., 2012) and the flasks incubated in a Tecnal shaker TE 421 (São Paulo, SP, Brazil) at 30 °C with agitation at 120 rpm. The mean culture time was 192 h, and an aliquot of 15 mL was removed every 24 h for the following analyses: biomass, reducing sugars in maltose, pigments and the color parameters.

2.3.3. Determination of the substrate, biomass and pigments

Aliquots of 15 mL were removed every 24 h and filtered through Whatman nº 1 quantitative filter paper (Madiston, England). The biomass was quantified by weighing and the filtrate used to quantify the reducing sugars by the 3,5-dinitrosalicylic acid method (Miller, 1959) and quantify the pigments on spectrophotometry (Cirrus 80, Piracicaba,

Table 2
Color parameters of the pigments produced by *Monascus ruber* CCT 3802 cultivated under different pH conditions in solid culture.

pH	Color				
	L^*	a^*	b^*	C^*	h°
2.0	71.11 ± 0.08^{a}	26.48 ± 0.39^{i}	115.8 ± 0.09^{a}	118.72 ± 0.01^{a}	77.11 ± 0.19^{a}
2.5	65.1 ± 0.36^{b}	35.69 ± 0.02^{h}	110.06 ± 0.01^{b}	110.37 ± 0.09^{b}	71.13 ± 0.01^{b}
3.0	5.36 ± 0.55^{c}	44.73 ± 0.07^{g}	59.43 ± 0.33^{c}	74.38 ± 0.30^{g}	53.03 ± 0.11^{c}
3.5	51.66 ± 0.16^{d}	50.57 ± 0.41^{f}	51.25 ± 0.28^{f}	82.02 ± 0.49^{h}	45.38 ± 0.07^{d}
4.0	38.83 ± 0.10^{h}	63.39 ± 0.01^{a}	57.78 ± 0.15^{d}	85.77 ± 0.11^{c}	42.34 ± 0.07^{e}
4.5	48.29 ± 0.07^{e}	57.88 ± 0.01^{e}	46.28 ± 0.22^{i}	74.10 ± 0.15^{g}	38.64 ± 0.12^{h}
5.0	47.55 ± 0.45^{ef}	58.58 ± 0.43^{de}	51.13 ± 0.14^{f}	77.45 ± 0.42^{e}	41.11 ± 0.13^{f}
5.5	46.73 ± 0.70^{f}	60.48 ± 0.39^{bc}	49.43 ± 0.49^{g}	78.10 ± 0.61^{e}	39.25 ± 0.09^{g}
6.0	45.75 ± 1.29^{g}	61.50 ± 0.24^{b}	54.34 ± 0.31^{e}	76.28 ± 0.38^{d}	41.46 ± 0.05^{f}
6.5	45.98 ± 2.17^{e}	59.47 ± 0.43^{cd}	47.78 ± 0.15^{h}	76.28 ± 0.43^{f}	38.77 ± 0.11^{h}
7.0	47.92 ± 0.07^{e}	58.86 ± 0.11^{de}	46.82 ± 0.07^{hi}	75.21 ± 0.13^{fg}	38.50 ± 0.01^{h}

L^*: luminosity (pure white to pure black); a^*: intensity of green (−) and red (+); b^*: intensity of blue (−) and yellow (+); C^*: chroma; h°: hue angle. Means followed by the same letter in the same column do not differ significantly according to Tukey's test at a level of significance of $p \leq 0.05$).

SP, Brazil) at 410 and 510 nm for yellow and red pigments, respectively (Vendruscolo et al., 2016).

2.3.4. Thermal stability

The thermal stability of the pigments was determined in a 500 mL jacketed glass reactor containing 200 mL of the pigment solution with the absorbance adjusted to approximately 1.0 unit of absorbance at 510 and 410 nm (~1.0 AU_{nm}). The temperature was controlled by circulating the water as from a Microquímica MQBMP-01 heat controlled water bath (Palhoça, SC, Brazil) (Vendruscolo et al., 2013). The stability was determined in the range from 50 to 90 °C, estimating the thermal degradation constant (D_C), the half-life ($t_{1/2}$) and the activation energy (E_a). The thermal degradation constant was estimated using the first order kinetic model shown in Equation (4).

$$\frac{dA}{dt} = -D_C . t \tag{4}$$

where A was the absorbance (AU_{410nm} or AU_{510nm}), t the time (h) and D_C the thermal degradation constant (h^{-1}). Equation (4) can be linearized using the contour conditions: $A = A_0$ when $t = 0$ and $A = A$ when $t = t$; resulting in Equation (5).

$$\ln\left(\frac{A}{A_0}\right) = D_C . t \tag{5}$$

where A is the absorbance at time t ($AU_{510\ nm}$) and A_0 is the initial absorbance ($AU_{510\ nm}$). The half-life ($t_{1/2}$) was calculated for each condition studied as from the values for D_C by way of Equation (6) where A/A_0 was equal to 2.

$$t_{1/2} = \frac{\ln 2}{D_C} \tag{6}$$

where $t_{1/2}$ is the half-life (h). The energy of activation (Ea) of the pigments was determined using the Arrhenius model according to Equation (7).

$$D_C = D_0 . e^{\left(\frac{-E_a}{RT}\right)} \tag{7}$$

where Ea is the activation energy (Kcal mol^{-1}), D_0 the pre-exponential factor (h^{-1}), R is the universal gas constant (cal mol^{-1} K^{-1}) and T is the temperature (K). Equation (8) was obtained by linearizing the Arrhenius model.

$$\ln D_C = -\frac{E_a}{R} . \left(\frac{1}{T}\right) + \ln D_0 \tag{8}$$

The value of the activation energy (Ea) was obtained from the linear regression of the angular coefficient of the values for the natural logarithm of D_C as a function of the inverse of the absolute temperature (in Kelvin), multiplied by the ideal gas constant (1.987 cal mol^{-1} K^{-1}), methodology proposed by Vendruscolo et al. (2013).

2.4. Statistical analysis

The analysis of variance (ANOVA) and Tukey's test at a significance level of 5% ($p < 0.05$) were used to confirm the arithmetic means, using the software Statistica 7.1.

3. Results and discussion

3.1. Solid state growth

Fig. 1 and Table 1 show the responses with respect to the evolution of the radius of the *Monascus ruber* colonies as a function of culture time, and also information concerning the visual aspect of the colonies cultivated at different pH values. It can be seen that the fastest radial growth velocity was obtained in the culture at pH 6.5 (0.0888 mm h^{-1}), followed by that obtained at pH 7.0 (0.0883 mm h^{-1}), whereas the slowest radial growth velocity (0.0434 mm h^{-1}) was obtained when cultivated at pH 2.0, clearly showing the influence of the pH value on the radial growth of *Monascus ruber*, which also made the visual aspect vary (Fig. 3). It can be seen that *Monascus ruber* colonies cultivated at pH 2.0 and 2.5 showed the formation of yellow pigments, those cultivated at pH 3.0 and 3.5 orange pigments, and those cultivated at pH values above 4.0 produced red pigments.

The radial growth velocities of the colonies cultivated at pH 2.0 were statistically different from all the others ($p \leq 0.05$). The greatest radial growth velocity was obtained when the microorganism was cultivated at pH 6.5, although it was not statistically different from the other velocities, except when compared with those obtained below pH 3.0.

Velmurugan et al. (2011) observed that pH was one of the most important factors in microbial growth and in the metabolic activity of *Monascus purpureus* during solid fermentation. It can be seen that fungal growth was lower at pH 1.0 and 2.0 but the growth velocity was greater at pH 3.0 and 4.0. Shi et al. (2015) reported that the lowest substrate pH value used promoted the synthesis of yellow pigments, whereas a higher pH value resulted in red pigments.

Fig. 2 shows the spectrophotometric scans of the pigments produced by *Monascus ruber*, where it can be seen that all the scans presented an absorbance peak at 400 nm. In the cultures carried out at pH values above 3.0, the orange and red colors superimposed on the yellow color, the predominant color being that of the pigment with the greatest colorific power. The scans of the pigments of the colonies cultivated at pH 2.0 and 2.5 only presented a peak of the yellow pigment (400 nm), whereas the pigments produced in cultures at pH 4.0 and above also presented a peak at 510 nm, characteristic of the red pigment.

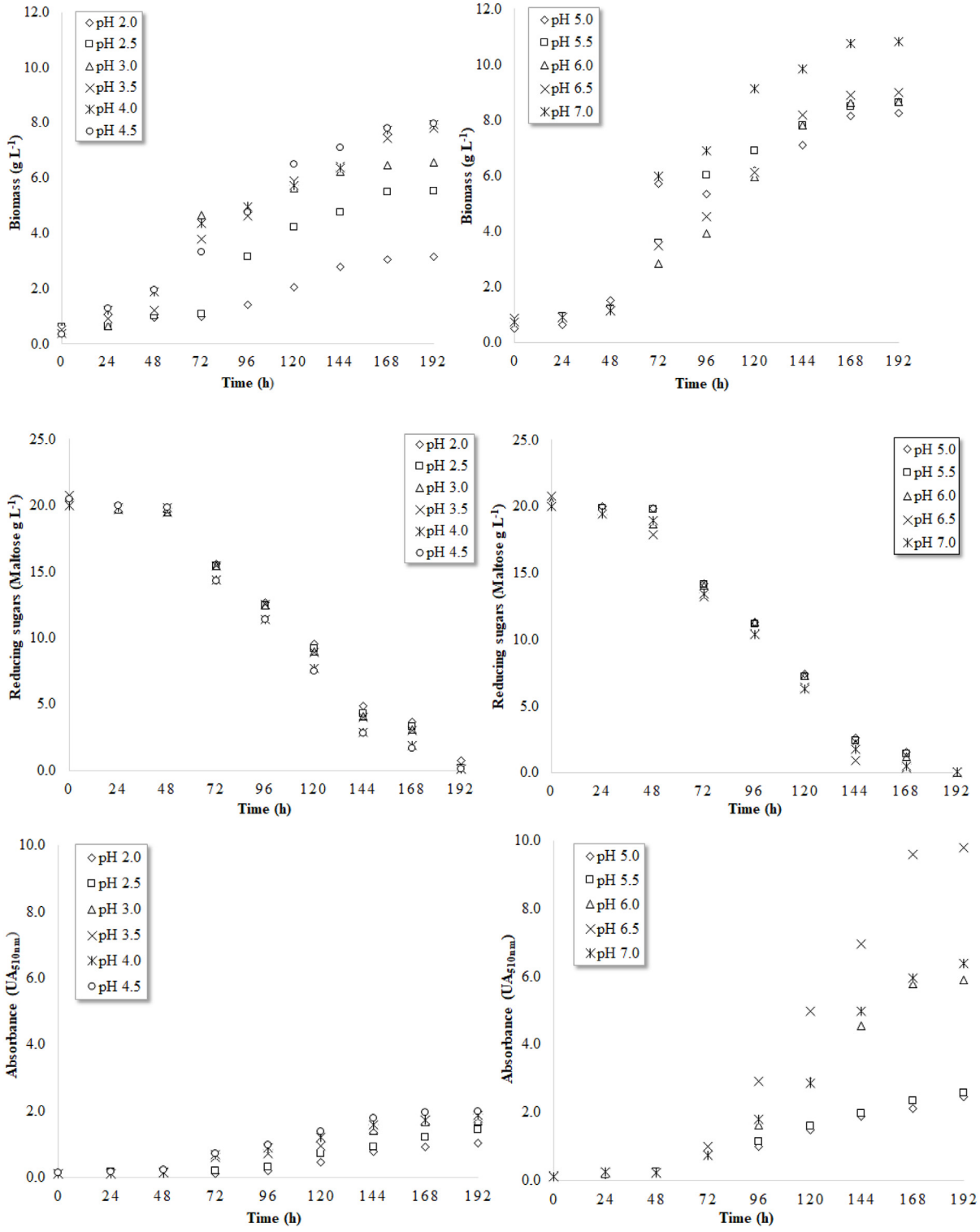

Fig. 4. *Monascus ruber* CCT 3802 cultivated under different pH conditions (a) biomass, (b) substrate and (c) pigments.

Velmurugan et al. (2011) also observed that the cultivation of *Monascus ruber* at lower pH values presented peaks at 400 nm and that at higher pH values presented a peak at 510 nm as well as that at 400 nm.

Table 2 shows the color parameters of the pigments produced under different pH conditions, the color characteristics of the samples mostly depending on the color and concentration of the dominant pigment in the mixture. For cultures at pH 2.0 and 2.5 the yellow pigments were dominant and the values for luminosity were 71.11 to 65.41, and those for $b \times$ from 115.8 to 110.06, corresponding to the yellow color. For cultures at pH 3.0 and 3.5 the parameters for L^* were from 56.36 to 51.66 and those for $b \times 59.43$ to 51.25, corresponding to a medium orange color. On the other hand at pH values above 4.0 red pigments were produced and low amounts of yellow pigments, with values for $a \times$ of 63.39 to 58.86. Shi et al. (2015) evaluated the production of *Monascus* pigments under different pH conditions and observed the formation of orange pigments at pH 2.0 and 4.0, whereas at pH 6.5 red pigments were produced with only small amounts of yellow pigments with a hue angle of 22.80, corresponding to dark red, confirming that

Table 3
Color parameters of the pigments produced by *Monascus ruber* CCT 3802 cultivated under different pH conditions (2.0–7.0) in submerged culture.

pH	CIELAB Color				
	*L**	*a**	*b**	*C**	*h°*
2.0	73.50 ± 0.07^{a}	22.33 ± 0.04^{J}	73.39 ± 0.02^{a}	76.77 ± 0.39^{cd}	73.08 ± 0,01^{a}
2.5	63.29 ± 0.38^{b}	28.64 ± 0.05^{i}	66.27 ± 0.07^{b}	72.41 ± 0.12^{ef}	66.64 ± 0,07^{b}
3.0	60.81 ± 0.07^{c}	34.71 ± 0.07^{h}	62.38 ± 0.40^{c}	71.32 ± 0.14^{fg}	60.48 ± 0,38^{c}
3.5	59.28 ± 0.23^{d}	37.66 ± 0.07^{g}	59.45 ± 0.45^{d}	70.21 ± 0.07^{g}	57.43 ± 0.17^{d}
4.0	57.67 ± 0.03^{e}	41.39 ± 0.08^{f}	57.56 ± 0.02^{e}	71.5 ± 0.02^{f}	54.45 ± 0.01^{e}
4.5	54.44 ± 0.16^{f}	50.82 ± 0.09^{e}	57.60 ± 0.02^{e}	76.66 ± 0.01^{c}	48.71 ± 0.02^{f}
5.0	54.45 ± 0.01^{f}	52.49 ± 0.53^{d}	53.87 ± 0.04^{f}	75.37 ± 0.04^{d}	45.57 ± 0.13^{g}
5.5	44.72 ± 0.06^{g}	56.67 ± 0.31^{c}	45.82 ± 0.09^{g}	72.81 ± 0.19^{e}	38.76 ± 0.03^{h}
6.0	38.88 ± 0.14^{h}	66.70 ± 0.02^{b}	43.39 ± 0.24^{h}	79.61 ± 0.04^{b}	33.07 ± 0.06^{i}
6.5	24.67 ± 0.12^{j}	75.55 ± 0.04^{a}	39.71 ± 0.04^{i}	85.47 ± 0.03^{a}	27.63 ± 0.02^{j}
7.0	26.62 ± 0.22^{i}	67.22 ± 0.07^{b}	44.34 ± 0.31^{h}	80.41 ± 0.12^{b}	33.55 ± 0.07^{i}

*L**: luminosity (pure white to pure black); *a**: intensity of green (−) and red (+); *b**: intensity of blue (−) and yellow (+); *C**: chroma; *h°*: hue angle. Means followed by the same letter in the same column do not differ significantly according to Tukey's test at a level of significance of $p \leq 0.05$).

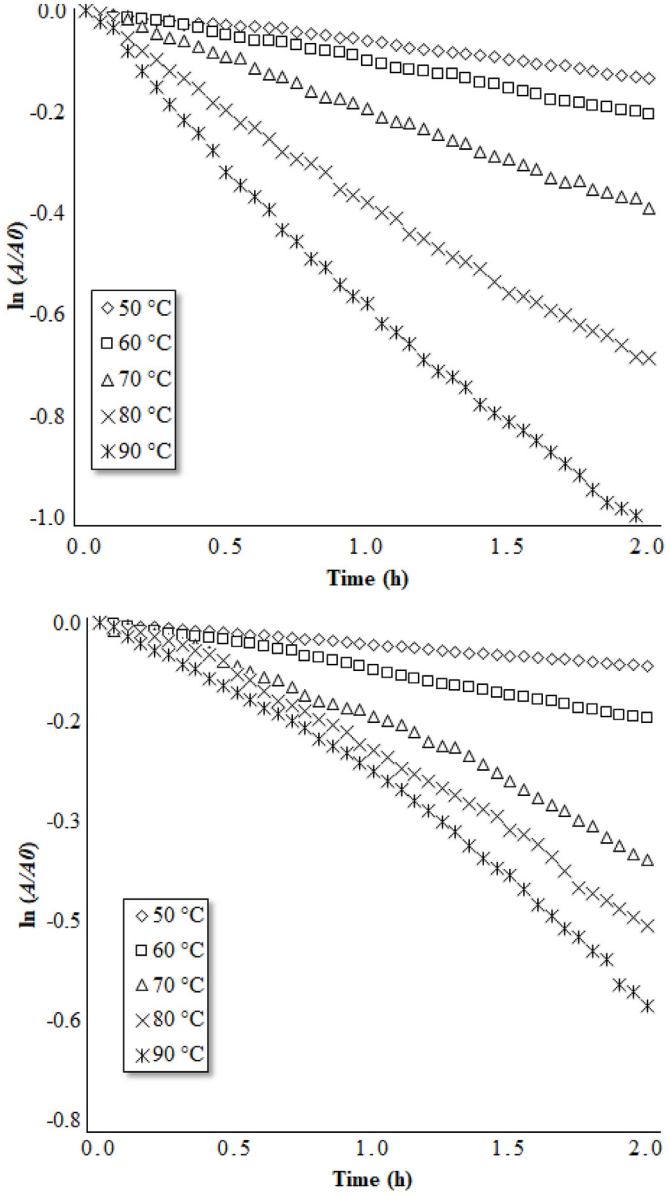

Fig. 5. Thermal degradation of the red and yellow pigments produced in *Monascus ruber* CCT 3802 cultures.

Table 4
D_C, $t_{1/2}$ and *Ea* of the pigments under the different thermal treatments applied.

Pigment	Temperature (°C)	D_C (h^{-1})	$t_{1/2}$ (h)	*Ea* (Kcal mol^{-1})
Red	50	0.0008 ± 0.022	14.4405 ± 0.131	13.735
	60	0.0018 ± 0.011	6.4180 ± 0.210	
	70	0.0033 ± 0.032	3.5007 ± 0.122	
	80	0.0058 ± 0.025	1.9918 ± 0.023	
	90	0.0084 ± 0.121	1.3752 ± 0.162	
Yellow	50	0.0327 ± 0.001	21.1971 ± 0.154	12.851
	60	0.0433 ± 0.056	16.0080 ± 0.002	
	70	0.1785 ± 0.012	3.8831 ± 0.230	
	80	0.2357 ± 0.034	2.9408 ± 0.0125	
	90	0.2851 ± 0.022	2.4312 ± 0.1003	

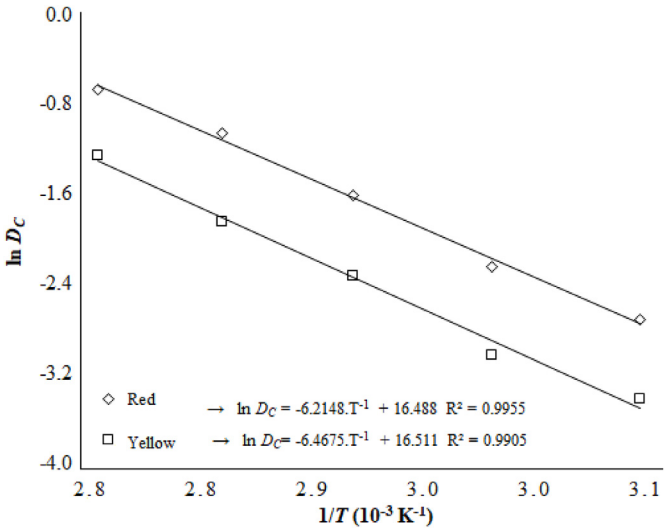

Fig. 6. Determination of the activation energy (*Ea*) of the pigments produced by *Monascus ruber* CCT.

observed in the present study.

3.2. Submerged fermentation

Fig. 4 shows the results obtained for the submerged cultures of *Monascus ruber* under different pH conditions. The culture carried out at pH 2.0 had the longest lag phase (~72 h) with the production of a yellow pigment, soluble in the culture medium. The cultures carried out at pH 3.0 and 3.5 produced an orange pigment with low solubility in the culture medium and the cultures carried out at pH values above 4.0

produced red pigments.

The existence of differences in the evolution of biomass concentration and pigments according to the pH value was verified, and at the same time the variation in absorbance was visible. Fig. 3 clearly shows the different colors of the cultures, highly acid values (pH 2.0 and 2.5) producing yellow pigments, intermediate values (pH 3.0 and 3.5) producing orange pigments, and pH values above 4.0 producing red pigments. These results confirmed the results produced in solid media. Vendruscolo et al. (2016) observed that changes in the pH of submerged cultures modified the color of the culture medium in *Monascus ruber* CCT 3802 cultures.

Table 3 shows the color parameters of the pigments. Yellow pigments were predominant in cultures carried out at pH 2.0 and 2.5, as in the solid medium cultures. The L* values of the cultures varied from 73.5 to 63.29 and the $b \times$ values from 73.39 to 66.27, corresponding to a yellow color. The values for L^* of the cultures carried out at pH 3.0 and 3.5 were between 60.81 and 58.28 and the values for $b \times$ from 62.38 to 59.45, corresponding to a medium to light orange color. The cultures developed with pH values above 4.0 presented an intense red color, with values for $a \times$ varying from 41.39 to 67.22, values for $b \times$ between 57.56 and 44.34, and values for L^* varying between 57.67 and 26.62, corresponding to medium to dark red samples. It should be pointed out that the increase in pH from 2.0 to 7.0 resulted in a linear decrease in the hue angles of the cultures from 73.08 to 33.55, corresponding to a change in color from yellow to red, confirming that the cultures of *Monascus ruber* in maltose syrup under different pH conditions provided different colors.

Fig. 5 presents the behavior of ln *(A/A0)* versus heat treatment time and Table 4 the kinetic parameters of D_C and $t_{1/2}$ of the pigments. It can be seen that the red pigments degraded quicker than the yellow pigments. Greater stability was obtained at low pH values, since the yellow pigment was produced in cultures at pH 2.0 and 2.5. These results clearly showed that the half-life of the yellow pigment was greater than that of the red pigment. Linear regression was obtained, demonstrating that degradation of the pigments followed a first order kinetic model with a good linear regression coefficient ($0.97 < R^2 > 0.99$). The same behavior was observed by Vendruscolo et al. (2013) for the orange and red pigments of *Monascus ruber* CCT 3802. Fernández-López et al. (2013) evaluated the thermal stability of aqueous solutions of natural red pigments (elderberry, red cabbage, hibiscus, red beetroot and red cochineal) in the range from 50 to 90 °C and observed that the red cochineal pigments exhibited the greatest thermal stability amongst the natural dyes after being submitted to 6 h at 50 °C, with a half-life of 5.52 h, demonstrating that the yellow and red pigments of the present study were more stable to degradation than those reported in the literature.

Ou et al. (2009) showed that the monocholine K produced by *Monascus* in fermented products was more stable in the pH range from 2.0 to 3.0, but as the pH value increased to 11 so the monocholine K content decreased rapidly, and thus the authors concluded that monocholine K was more stable at low pH values, a fact also observed in the present study.

Fig. 6 showed that the experimental data fitted the Arrhenius model. When the angular coefficient ~ Ea/R of the linear regression was multiplied by 1.987 (ideal gas constant in cal mol^{-1} K^{-1}, a value of 13.735 was obtained for the *Ea* of the red pigment and of 12.85092 for the yellow pigment (Table 4).

The studies carried out by Levenspiel (1986) pointed out that reactions with high activation energies were much more sensitive to temperature, and that the Arrhenius model was the first adequate approximation to determine the effect of temperature on the kinetic equation, and also fitted the experimental results well in a wide temperature range, a behavior also verified in the present study.

4. Conclusions

Maltose syrup was shown to be a potential substrate for the production of pigments by *Monascus ruber* CCT 3802, potentiating the use of byproducts from the syrup processing industry to produce microbial pigments. The pH value exerted a significant influence on both the radial growth velocity and the production of pigments by *Monascus ruber*, making it possible to obtain different pigments as a function of the culture pH value. Both the pigments produced were resistant to heat treatment, such that they could be added to products submitted to heat treatment.

Conflicts of interest

The authors declare that there is no conflict of interest.

Acknowledgements

The authors gratefully acknowledge the Committee for Professional Training of Higher Education Staff (CAPES- Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), for the research scholarship granted and the *Instituto de Fosfatos Biológicos* (Goiânia, GO, Brazil) for donated of the maltose syrup residue.

References

Bühler, R.M.M., Dutra, A.C., Vendruscolo, F., Moritz, D.E., Ninow, J.L., 2013. *Monascus* pigment production in bioreactor using a co-product of biodiesel as substrate. Ciência Tecnol. Aliment. 33, 9–13. https://doi.org/10.1590/S0101-20612013000500002.

Cereda, M.P., Vilpoux, O., 2003. Tecnologia, usos e potencialidades de tuberosas amiláceas Latino Americanas. Fundação Cargill, São Paulo.

Chacón-Ordoñez, T., Cchweiggert, A., Bosy-Westphal, A., Jiménez, V.M., Carle, R., Esquivel, P., 2017. Carotenoids and carotenoid esters of orange- and yellow-fleshed mamey sapote (*Pouteria sapota* (Jacq.) H.E. Moore & Stearn) fruit and their postprandial absorption in humans. Food Chem. 221, 673–682. https://doi.org/10.1016/j.foodchem.2016.11.120.

Cordeiro, L.G., El-Aouar, A.A., Gusmão, R.P., 2012. Caracterização do bagaço de malte oriundo de cervejarias. Revista Verde de Agroecologia e Desenvolvimento Sustentável. 7, 20–22.

Dasan, B.G., Boyaci, I.H., Mutlu, M., 2017. Nonthermal plasma treatment of *Aspergillus* spp. spores on hazelnuts in an atmospheric pressure fluidized bed plasma system: impact of process parameters and surveillance of the residual viability of spores. J. Food Eng. 196, 139–149. https://doi.org/10.1016/j.jfoodeng.2016.09.028.

Domínguez-Santosa, R., Kasalková, K., Gárcía-Estrada, C., Barreiro, C., Ibanez, A., Morales, A., Martin, J.F., 2017. Casein phosphopeptides and $CaCl_2$ increase penicillin production and cause an increment in microbody/peroxisome proteins in *Penicillium chrysogenum*. J. Proteomics 5, 151–160. https://doi.org/10.1016/j.jprot.2016.12.021.

Dufossé, L., Galaup, P., Yaron, A., Arad, S.M., Blanc, P., Murthy, K.N.C., Ravishankar, G.A., 2005. Micro-organisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality? Trends Food Sci. Technol. 16, 389–406. https://doi.org/10.1016/j.tifs.2005.02.006.

Fernández-López, J.A., Angosto, J.M., Giménez, P.J., León, G., 2013. Thermal stability of selected natural red extracts used as food colorants. Plant Foods Hum. Nutr. 68, 11–17. https://doi.org/10.1007/s11130-013-0337-1.

Hilares, R.T., Souza, R.A., Marcelino, F.M., Silva, S.S., Dragone, G., Mussatto, S.L., Santos, J.C., 2018. Sugarcane bagasse hydrolysate as a potential feedstock for red pigment production by *Monascus ruber*. Food Chem. 245, 786–791. https://doi.org/10.1016/j.foodchem.2017.11.111.

Kang, B., Zhang, X., Wu, Z., Qi, H., Wang, Z., 2013. Effect of pH and nonionic surfactant on profile of intra cellular and extracellular *Monascus* pigments. Process Biochem. 48, 759–767. https://doi.org/10.1016/j.procbio.2013.03.020.

Lee, C.L., Wen, J.Y., Hsu, Y.W., Pan, T.M., 2016. The blood lipid regulation of *Monascus* produced monascin and ankaflavin via the suppression of low-density lipoprotein cholesterol assembly and stimulation of apolipoprotein A1 expression in the liver. J. Microbiol. Immunol. Infect. 4, 1–11. https://doi.org/10.1016/j.jmii.2016.06.003.

Levenspiel, O., 1986. Ingenieria de las Reacciones Químicas. Editorial Reverté S. A., Barcelona, Espanã. vol. 2. pp. 634.

Long, N.N.V., Vasseur, V., Coroller, L., Dantigny, P., Pance, S.L., Weill, A., Mounier, J., Rigalma, K., 2017. Temperature, water activity and pH during conidia production affect the physiological state and germination time of *Penicillium* species. Int. J. Food Microbiol. 241, 151–160. https://doi.org/10.1016/j.ijfoodmicro.2016.10.022.

Lv, J., Zhang, B., Liu, X., Zang, C., Chen, L., Xu, G., Cheung, C.K., 2017. Enhanced production of natural yellow pigments from *Monascus purpureus* by liquid culture: the relationship between fermentation conditions and mycelial morphology. J. Biosci. Bioeng. 124, 452–458. https://doi.org/10.1016/j.jbiosc.2017.05.010.

Marioa, D.A., Santos, R.C.V., Denardi, L.B., Vaucher, R.A., Santurioa, J.M., Alves, S.H., 2016. Interference of melanin in the susceptibility profile of *Sporothrix* species to

amphotericin B. Rev. Iberoam. De. Micol. 33, 21–25. https://doi.org/10.1016/j.riam.2015.03.001.

Miller, G.L., 1959. Use of dinitrosalicilic acid reagent for determination of reducing sugar. Anal. Chem. 31, 426–428. https://doi.org/10.1021/ac60147a030.

Ning, Z.Q., Cui, H., Xu, Y., Huang, Z.B., Tu, Z., Li, Y.P., 2017. Deleting the citrinin biosynthesis-related gene, ctnE, to greatly reduce citrinin production in *Monascus aurantiacus* Li AS3.4384. Int. J. Food Microbiol. 241, 325–330. https://doi.org/10.1016/j.ijfoodmicro.2016.11.004.

Ou, H.P., Wang, C.C.R., Lai, L.S., 2009. Thermal degradation kinetics analysis of monacolin K in *Monascus*-fermented products. Food Sci. Technol. 42, 292–296. https://doi.org/10.1016/j.lwt.2008.05.021.

Savadkoohi, S., Hoogenkamp, H., Shamsi, K., Farahnaky, A., 2014. Color, sensory and textural attributes of beef frankfurter, beef ham and meat-free sausage containing tomato pomace. Meat Sci. 97, 410–418. https://doi.org/10.1016/j.meatsci.2014.03.017.

Shi, k., Song, D., Chen, G., Pistolozzi, M., Wu, Z., Quan, L., 2015. Controlling composition and color characteristics of *Monascus* pigments by pH and nitrogen sources in submerged fermentation. J. Biosci. Bioeng. 120, 145–154. https://doi.org/10.1016/j.jbiosc.2015.01.001.

Velmurugan, P., Hur, H., Balachandar, V., Kamala-Kannan, S., Lee, Kui-Jae, Sang-Myung, L., Jong-Chan, C., Shea, P.J., Byung-Taek, O., 2011. *Monascus* pigment production by solid-state fermentation with corn cob substrate. J. Biosci. Bioeng. 112, 590–594. https://doi.org/10.1016/j.jbiosc.2011.08.009.

Vendruscolo, F., Muller, B.L., Moritz, D.E., Oliveira, D., Schmidell, W., Ninow, J.L., 2013. Thermal stability of natural pigments produced by *Monascus ruber* in submerged fermentation. Biocatalysis. Agric. Biotechnol. 2, 278–284. https://doi.org/10.1016/j.bcab.2013.03.008.

Vendruscolo, F., Schmidell, W., Moritz, D.E., Rose, M.M.B., Oliveira, D., Ninow, J.L., 2016. Isoelectric point of amino acid: importance for *Monascus* pigment production. Biocatalysis. Agric. Biotechnol. 5, 179–185. https://doi.org/10.1016/j.bcab.2015.12.006.

Wana, Y., Zhang, J., Hana, H., Liua, Y., Gao, M., 2016. Citrinin-producing capacity of *Monascus purpureus* in response to low − frequency magnetic fields. Process Biochem. 11, 1–5. https://doi.org/10.1016/j.procbio.2016.11.009.

Wanga, Y., Pang, J., Zheng, Y., Jiang, P., Gong, W., Chenc, X., Chena, D., 2017. Genetic manipulation of the bifunctional gene, carRA, to enhance lycopene content in *Blakeslea trispora*. Biochem. Eng. J. 119, 27–33. https://doi.org/10.1016/j.bej.2016.12.011.

Wu, P.W., 2015. A review on the analysis of ingredients with health care effects in health food in Taiwan. J. Food Drug Anal. 23, 343–350. https://doi.org/10.1016/j.jfda.2015.03.007.

Xu, B.J., Jia, X.Q., Gu, L.J., Sung, C.K., 2006. Review on the qualitative and quantitative analysis of the mycotoxin citrinin. Food Control 17, 271–285. https://doi.org/10.1016/j.foodcont.2004.10.012.

Yuliana, A., Singgih, M., Julianti, E., Blanc, P.J., 2017. Derivates of azaphilone *Monascus* pigments. Biocatalysis. Agric. Biotechnol. 9, 183–194. https://doi.org/10.1016/j.bcab.2016.12.014.

Zhang, B.B., Lu, L.P., Xu, G.R., 2015. Why solid-state fermentation is more advantageous over submerged fermentation for converting high concentration of glycerol into Monacolin K by *Monascus purpureus* 9901: a mechanistic study. J. Biotechnol. 26, 60–65. https://doi.org/10.1016/j.jbiotec.2015.04.011.

Zhang, B., Xing, H., Jiang, B., Chen, L., Xu, G., Jiang, Y., Zhang, D., 2018. Using millet as substrate for efficient production of monacolin K by solid-state fermentation of *Monascus ruber*. J. Biosci. Bioeng. 125, 333–338. https://doi.org/10.1016/j.jbiosc.2017.10.011.