ASIAN JOURNAL OF BIOMEDICAL & PHARMACEUTICAL SCIENCES

e-ISSN: 2249-622X

RESEARCH ARTICLE

Improving the Production and CIELAB* Color Parameters of *Monascus ruber* Pigments using a Fractional Factorial Design

Cristiane Cardoso Correia Teixeira; Guilherme Augusto Teixeira & Luis Alexandre Pedro Freitas*

School of Pharmaceutical Sciences at Ribeirão Preto

University of São Paulo

Via do Café s/n, Campus USP, Ribeirão Preto, SP, 14040-903, Brazil

4th Dec 2012
Received in revised form:
18th Dec 2012
Accepted:
20th Dec 2012
Available online:
15th Jan 2013

Online ISSN 2249–622X
http://www.jbiopharm.com

ABSTRACT

The aim of this work was to study the production of pigments by M*onascus ruber* Van Tiegham using submerged culture technique. The fermentation media contained rice flour, peptone, glycerin, glucose, $MgSO_4$, KNO_3 and the variables studied included the addition of zinc, ammonium and glutamate in the liquid culture. The experiments were designed to investigate the production of pigments using a Box Behnken design. The influence of each of these factors on pigment production were evaluated by the ratio of 500 nm and 400 nm absorbance measurements and by the color parameters of the *Commission Internationale d'Eclairage* L*A*B* system. Total and red pigments measured as absorbance units showed optical density values as high as 21.78 and 11.67 absorbance units, respectively, which was close to values reported in the literature for solid-state culture. The response surface analysis proved a quadratic effect of zinc concentration on both pigment production and color quality. This result is quite important because the quadratic effect of zinc was not reported before in the literature and it demonstrates there is an optimum concentration of zinc for pigment production and quality. More importantly, color L*A*B* measurements showed that the increase in total red pigment production caused no prejudice in color quality within the range of the experimental conditions. The results demonstrate the feasibility of pigment production with high color quality in submerged culture.

Keywords: Red pigments, submerged culture, CIELAB* color space, Box-Behnken designe.

1. INTRODUCTION

The recent scientific finding that most synthetic colorants have carcinogenic and teratogenic effects has raised growing interest in natural colorants and resulted in many ongoing studies (*1-3*). One promising alternative is the production of colorants by microorganisms, which have been used by man since ancient times. The first history of success in the industrial production of pigments using microorganisms was in the Europe in the production of β-carotene by the yeast *Blakeslea*, in 1995. The β-carotene produced by this biotechnology is chemically equivalent to that produced by a synthetic route and is widely accepted as a food colorant (*4*).

Currently, natural colorants or pigments are of emerging importance in industry, particularly in food production where color is an important determinant in organoleptic quality (*5,6*).

Monascus strains were discovered by van Tiegham (1884) and were known in Europe as a contaminant in cereals, starch and silage (*7*). In Asia, *Monascus sp.* has been used for many years to prepare pigments as well as fermented foods and beverages (*8*). The red pigments produced by organisms found in the rice grains, such as red mold rice, red yeast rice, red koji or anka, are the most widely used in Asia, mainly for coloring fermented foods (*9*), and the species are also known as red mold rice, red yeast rice,

*Corresponding author: Luis Alexandre Pedro Freitas, | School of Pharmaceutical Sciences at Ribeirão Preto, University of São Paulo
Via do Café s/n, Campus USP, Ribeirão Preto, SP, 14040-903, Brazil| Email: lapdfrei@usp.br

red koji or anka (*10-12*). Red mold rice is now used as a popular natural colorant and a dietary supplement worldwide (*13*). Although it has no industrial application, its secondary metabolites have anticholesteremic and antibacterial activities (*14-17*), which makes red mold rice a valid functional food candidate (17)

The coloring components obtained from *Monascus sp*. include at least six chemicals: rubropunctamine and monascorubramine (red-colored); rubropunctatin and monascorubrin (orange-colored); and monascin and ankaflavin (yellow-colored). These pigments are liposoluble and unstable under conditions of extreme pH (<2 and >14), heat or light (*19*). These pigments are present as a mixture in fermentation broth, and the isolation of red, orange and yellow pigments is not economically feasible, although the red pigments have the highest commercial value (*20-21*). The changing proportions of these main chemicals may give the different colors or shades to this natural pigment.

Some studies have been carried out to increase the red pigment production by *Monascus ruber* (*6,9,22,23*), since this monascus strain does not produce citrinin (24, 25). However, the majority of these reports used solid fermentation. In solid state fermentation, the red pigment production is supposedly greater than liquid state fermentation due to the diffusion of intracellular pigments in the solid matrix, which could increase the economic viability of this method (5, *26*). However, yields of red pigments are still too low to allow industrial scale production (*27*). Therefore, red pigment production by *Monascus ruber* in submerged culture is an interesting alternative (*28*). Submerged culture fermentation is widely applied in industrial biotechnology due to its reliable homogenization, allowing uniform conditions for the microorganism growth and fine control of culture factors such as pH, dissolved oxygen, temperature, agitation speed and nutrient concentration (*22,27*).

The aim of the present work was to investigate the effects of culture media additives on the production and ratio of red and yellow pigments by M*onascus ruber* Van Tieghem in submerged culture using response surface methodology. The pigment production was characterized by the final yield of red pigments, and the red-to-yellow pigment ratios were determined spectrophotometrically and by using the CIELAB* color space measurements. The experiments were carried out following a factorial design (Box-Behnken), and data were analyzed by response surface methodology.

2. MATERIAL AND METHODS

Microorganisms, culture media and conditions

The strain of *Monascus ruber* van Tiegham (alt. *Basipetospora rubra* Cole & Kendrick), Tax (79, 373), supplied by Fundação André Tosello de Pesquisa e Tecnologia (Campinas, Brazil), was preserved by spore growth in PDA medium with immobilization in silica beads and further drying under vacuum. The silica-immobilized strain was stored at 4ºC. Before transfer to fermentation flasks, *M. ruber* was seed cultured by extracting from silica using PDA medium and cultivated for 10 days. The inocula was prepared by homogenizing 16.8 g of *Monascus* mycelia wet mass in 10 mL of sterilized distilled water, followed by filtration in a sterilized cloth, which yield 2 mL of a suspension with 5.84 x 10^6 spores/mL. Fermentation was carried out by adding 2 mL of the *Monascus* suspension to 50 mL of a liquid medium containing rice flour at a concentration of 30 g/L, peptone 9 g/L, glycerin 30 g/L, glucose 110 g/L, $MgSO_4$ 1 g/L, KNO_3 2 g/L. The culture medium pH was adjusted to 6.5 ±0.1 using a Digimed pHmeter (Digimed Ltd., São Paulo, Brazil). The fermentation was completed in 250-mL Erlenmeyer flasks stored in air baths, model LM/AB 15 (Labmaq Ltd, Ribeirão Preto, Brazil), which have 15 magnetic stirring spots with digital controls for both temperature and stirring speed. The temperature was maintained at 30 ± 0.5ºC by a digital PID controller, and fermentation was completed under anaerobic conditions. The fermentation process was carried out for 7 days, after which the fermentation broth was filtered with a 0.45-mm membrane (Millipore Co., Billerica, USA) under vacuum and the mycelia were separated from the filtrate. Afterwards, the mycelia were ground in a mortar, ultrasonicated in a sonicator DES 500 (Unique Ltd., Indaiatuba, Brazil) for 5 minutes and then added to 100 mL of analytical purity ethanol. This suspension was magnetically stirred for 48 hours in the dark. The suspension was filtered in 0.22 µm membrane filter (Millipore Co., Billerica, USA) and added to the filtrate to obtain the final extract. The samples were immediately used or diluted for spectrophotometer and color space analysis.

Box-Behnken design

The experimental replicates followed a fractional factorial, Box-Behnken design (*29*) in order to verify the effect of three factors on the red and total pigment production by *Monascus*. The effects studied were addition of zinc, monosodium glutamate and ammonium to the fermentation medium. This experimental design allowed the investigation of the linear and quadratic effects of these factors on the pigments produced, as well as the possible interaction between these factors. The levels of pigments were determined spectrophotometrically and by color space analysis. The studied factors and their real and coded levels are shown in Table 1, while the complete factorial design is shown in Table 2. To analyze the levels adopted in this design, the factors studied needed to be decoded. The decoding formula is given by:

© Asian Journal of Biomedical and Pharmaceutical Sciences, all rights reserved.

$$Coded \cdot Variable = \frac{(uncoded \cdot value - 0.5 \times (high \cdot value + low \cdot value))}{0.5 \times (high \cdot value - low \cdot value)} \quad [1]$$

Some of the effects were modeled by a response function applied as a quadratic equation, fitted by response surface methodology as given below:

$$Y_i = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_4X_1^2 + A_5X_2^2 + A_6X_3^2 + A_7X_1X_2 + A_8X_1X_3 + A_9X_2X_3 \quad [2]$$

Factors	Levels		
	+1	0	-1
$ZnSO_4$, Zn (g/L)	0.8	0.4	0
Monosodium glutamate, MSG (g/L)	10	5	0
Ammonium, NH_4^+ (g/L)	2	1	0

Table 1 – Investigated factors and their levels in culture media

Exp.	Zn	MSG	NH_4^+
1	-1	-1	0
2	1	-1	0
3	-1	1	0
4	1	1	0
5	-1	0	-1
6	1	0	-1
7	-1	0	1
8	1	0	1
9	0	-1	-1
10	0	1	-1
11	0	-1	1
12	0	1	1
13	0	0	0
14	0	0	0
15	0	0	0

Table 2. Experimental design using the Box-Behnken method.

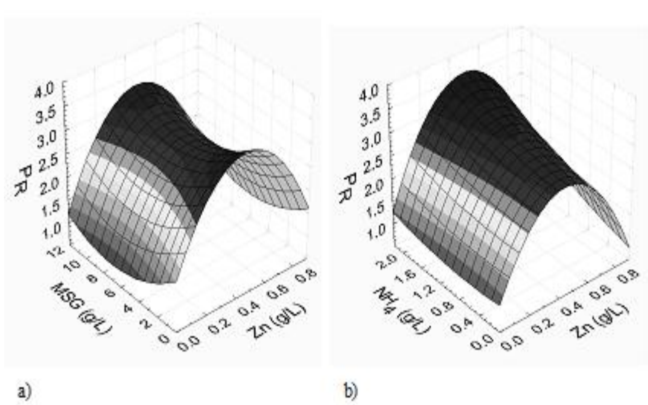


Figure 1 - Response surface of P_R as a function of: a) MSG and Zn; b) NH_4 and Zn.

where Y_i = dependent variable, X_1 = Zinc; X_2 = Monosodium glutamate; X_3 = ammonium; and A_i = polynomial coefficients.

The results were analyzed by multiple regression methods using the module Visual General Linear Model (VGLM) of the software Statistica 7 (Statsoft Inc., Tulsa, USA).

Pigment measurement

The determination of yellow and red pigments present in the extracts was made spectrophotometrically in a UV spectrophotometer model M330 (Camspec Co., Leeds, UK). Red pigment production was determined by measuring the units of absorbance per AU/cm. As shown in this table, P_R values ranged from 0.61 to 3.89 at the wavelength of 500 nm (*9*). The ratio of red to yellow pigments in the extract was proportional to the ratio of absorbencies in the wavelengths of 500 and 400 nm, respectively (*19,21,23*).

Color measurement

The color space indexes L*a*b* were measured by a "Cromameter" CR 200 colorimeter (Minolta Co. Ltd., Osaka, Japan). A 50 x 50 mm double layered white cotton cloth was submerged in 20 mL of the fermentation broth extract at 25°C for five minutes. The cloth was dried under a controlled 20 l/min air flow at room temperature. The procedure was repeated twice. The L*a*b* values were determined according to the CR200 manufacturer instructions. The CR 200 was calibrated with a standard white plate CR A44 (Minolta Co. Ltd., Osaka, Japan) at the start of each session. The parameters of color space were measured in duplicate samples using an observer angle of 0° and a measurement surface of 8 x 8 mm.

3. RESULTS AND DISCUSSION

The resulting red pigment production for this factorial design experiment was analyzed using the response surface technique (*29*). The optical densities of red pigments produced are given in units of absorbance per ml, P_R, in Table 3. As shown in this table, P_R values ranged from 0.61 to 3.89 AU/cm. However, as the culture media (50 ml) was extracted with 100 ml of ethanol, a dilution factor of 3 should be considered to compare our results with data of other authors, giving 1.83 to 11.67 AU/cm. Previously, 1 O.D. unit was considered to correspond to 15 mg/l of red pigments at 480 nm (*22*). However, the diversity of fermentation conditions makes comparison a difficult task as the concentrations of pigments are lower in submerged cultures than in solid state fermentation.

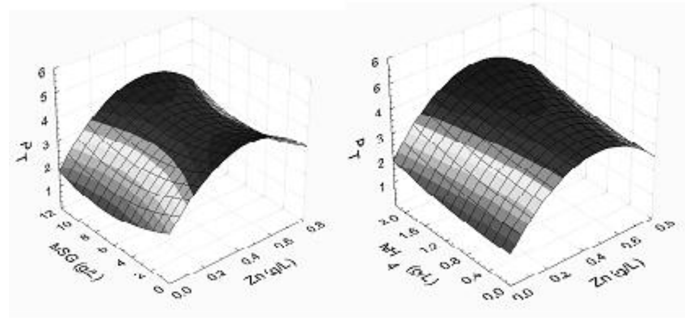
© Asian Journal of Biomedical and Pharmaceutical Sciences, all rights reserved.

For example, 25 O.D. units per gram of dry fermented substrate was achieved using jackfruit seeds as a substrate (*6*). The surface plots of red pigment production, P_R, represented by optical density at 500 nm are shown in Figure 1. The AU/cm readings for different zinc and ammonium contents are shown in Figure 1a. The absorbency at 500 nm increased with the addition of zinc until it reached a maximum level and then started to decrease. However, the surface analysis indicates that the red pigment production was not affected by the ammonium content in the culture media. The effect of monosodium glutamate addition on red pigment production is shown in Figure 1b. This figure presents the surface plot of AU/cm at 500 nm as a function of MSG and $ZnSO_4$ content. The greatest red pigment production is achieved with intermediary Zn levels, whereas the addition of MSG appears not to have an effect on pigment production. Based on these two surface plots, the only factor affecting the red pigment production was the $ZnSO_4$ content in culture media.

Exp.	Zn	MSG	NH_4^+	P_R	P_T	$A_{R/Y}$	a*/b*	L*
1	0	0	1	1.20	3.14	0.62	0.35	69.7
2	0.8	0	1	2.14	4.73	0.82	0.70	62.7
3	0	10	1	1.06	2.44	0.77	0.43	66.3
4	0.8	10	1	2.24	2.24	0.91	0.66	65.1
5	0	5	0	0.61	1.42	0.75	0.47	75.7
6	0.8	5	0	1.15	2.75	0.72	0.43	70.3
7	0	5	2	1.28	3.06	0.72	0.44	62.0
8	0.8	5	2	2.41	5.22	0.86	0.72	65.0
9	0.4	0	0	3.51	7.26	0.83	0.76	58.7
10	0.4	10	0	2.79	5.73	0.90	0.70	58.2
11	0.4	0	2	3.89	7.24	0.88	1.29	46.3
12	0.4	10	2	2.80	6.24	0.81	0.67	61.4
13	0.4	5	1	2.65	5.88	0.92	1.02	61.6
14	0.4	5	1	3.08	6.94	0.80	0.91	56.0
15	0.4	5	1	2.62	4.26	0.68	0.76	68.8

Table 3 - Characteristics of extracts containing the pigments

The total pigment, P_T, was obtained by the summation of O.D. measurements at the wavelengths of 400, 470 and 500 nm (Table 3 and Figures 2a and 2b). The P_T varied from 1.42 to 7.26 AU/cm, but after application of the dilution factor, they represent values of 4.26 to 21.78 AU/cm. It means that varying the experimental conditions P_T could be multiplied by a factor of 5.1. The surface plots of P_T as a function of ammonium and $ZnSO_4$ or MSG and $ZnSO_4$ concentrations are shown in Figures 2a and 2b, respectively. The shapes of these surfaces indicate that the characteristics of total pigment production were very similar to those of red pigment because in both cases, the curved behavior of the response surfaces indicates maximum pigment production at intermediate concentrations of zinc sulfate.



a) b)

Figure 2 - Response surface of P_T as a function of: a) MSG and Zn; b) NH_4 and Zn.

This observation is confirmed by the analysis of variance (ANOVA) on the experimental data of P_R and P_T. The ANOVA showed that $ZnSO_4$ affected P_R with a 5% significance, whereas the squared term of the Zn content influenced pigment production at a significance level of 0.1%, as shown in Table 4. The same statistical result was found for P_T, with the effects of Zn and its squared term significant at 5% and 0.1%, respectively. However, in the case of P_T, the ammonium concentration also influenced production, but with a significance level of 10%. The MSG content did not significantly influence either the P_R or the P_T. The best fits of response surface equations were obtained by multiple regression analysis considering only the significant factors. Equations 3 and 4 were obtained for P_R and P_T with R^2 = 0.712 and 0.936, respectively.

$$P_R = 2.59 + 0.47\left(\frac{Zn-0.4}{0.4}\right) - 1.29\left(\frac{Zn-0.4}{0.4}\right)\times\left(\frac{Zn-0.4}{0.4}\right) \quad [3]$$

$$P_T = 2.65 + 0.61\left(\frac{Zn-0.4}{0.4}\right) - 1.80\left(\frac{Zn-0.4}{0.4}\right)\times\left(\frac{Zn-0.4}{0.4}\right) \quad [4]$$

Factor	P (P_R)	P (P_T)	P ($A_{R/Y}$)	P(a*/b*)	P (L*)
Zn	0.022571*	0.021211*	0.155950	0.120821	0.489318
Zn^2	0.000154**	0.000074***	0.308223	0.007413****	0.029774*
MSG	0.327845	0.223271	0.414119	0.204781	0.386108
MSG^2	0.185296	0.161127	0.497697	0.898567	0.178017
NH_4	0.183427	0.128769	0.805485	0.144055	0.106002
NH_4^2	0.666764	0.674717	0.720949	0.718423	0.513675
Zn* MSG	0.822440	0.788491	0.76566	0.715050	0.590713
Zn * NH_4	0.697181	0.738978	0.413365	0.349987	0.447788
MSG *NH_4	0.928871	0.940912	0.495677	0.131163	0.186929
Error					

Table 4. Analysis of variance (ANOVA). Significance levels: *5%, **0.05%, ***0.001%, ****1%

© Asian Journal of Biomedical and Pharmaceutical Sciences, all rights reserved.

The proportion of red-to-yellow pigments was also been evaluated by the ratio of the respective absorbance measurement at 500 and 400 nm, $A_{R/Y}$. The values obtained for this ratio following the Box-Behnken experimental design are shown in Table 3. The surface plots in Figures 3a and 3b show the ratios as functions of ammonium, zinc and MSG contents in culture media. The surface in Figure 3a again indicates a curved or quadratic effect of zinc addition on $A_{R/Y}$, whereas there is an increase in this ratio with increasing addition of MSG. The same zinc effect is shown in Figure 3b, but the effect of ammonium seems to be more complex and interactive with zinc. However, the ANOVA performed on $A_{R/Y}$ data proved that none of these factors affected $A_{R/Y}$ at a 5% significance level, as shown in Table 4. Nevertheless, this statistical result does not indicate that zinc, ammonium and MSG do not affect *Monascus* pigment color, but rather that within the range of concentrations used in this study, the variability of the process was larger than the linear, quadratic and interaction effects of the factors. The negligible effects of these three factors on the $A_{R/Y}$ ratio indicates that the coloring quality of the pigments produced were not influenced by these additives. This is a positive finding because these results indicate that using the conditions applied in this experiment, the red pigment production may be improved without compromising pigment quality.

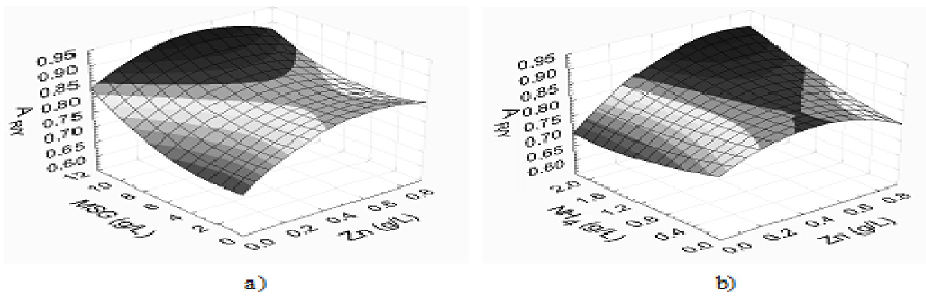


Figure 3 - Response surface of $A_{R/Y}$ as a function of: a) MSG and Zn; b) NH_4 and Zn.

From the standpoint of food colorants, *Monascus* products are better characterized by a color space criterion (*30*). The CIE (*Commission Internationale d'Eclairage*) L*a*b* (CIELAB) is one the most complete and accepted color space systems, with the main advantages of this system being the thorough description of colors visible to human eye and theoretical independence of L*a*b* from the measuring device.

The values of the a*/b* ratio for the 15 experiments performed in this study are presented in Table 3. The response surfaces for a*/b* are shown in Figures 4a and 4b. Figure 4a presents the surface plot of a*/b* as a function of MSG and $ZnSO_4$ contents in culture media. The surface indicates a non-linear effect of zinc on the a*/b* ratio. The a* and b* coordinates of the CIELAB represent the color positions between red and green as well as between yellow and blue, respectively. Positive values of a* indicate red, whereas the negative values represent green. The b* negative values correspond to blue, and the positive values indicate yellow. This indicates that high positive values of the ratio a*/b* correspond to predominantly red coloring, as desired for *Monascus* pigments. Figure 4b also presents the a*/b* response surface as functions of ammonium and Zn contents. The most important effect observed here is a quadratic increase in red pigment production with Zn addition. In both Figures 4a and 4b, the effects of MSG and ammonium seem to be insubstantial. This finding was confirmed by the ANOVA on a*/b* data, shown in Table 4, which verifies that the only significant effect, at a 1% level, was the squared term of $ZnSO_4$ content. It can also be asserted that the pigments obtained with intermediary Zn content had the highest quality when considering red pigment production as the final goal. Equation 5 was fitted to a*/b* data by multiple regression, taking into account only the significant terms and resulting in a correlation coefficient of $R^2 = 0.865$.

$$a^*/b^* = 0.0896 - 0.3508\left(\frac{Zn - 0.4}{0.4}\right)\times\left(\frac{Zn - 0.4}{0.4}\right) \quad [5]$$

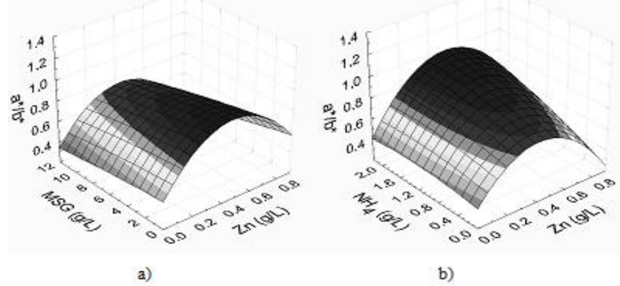


Figure 4 - Response surface of a*/b* as a function of: a) MSG and Zn; b) NH_4 and Zn.

© Asian Journal of Biomedical and Pharmaceutical Sciences, all rights reserved.

An equally important parameter in this experiment is the lightness of the color. The L* values for the *Monascus* pigments obtained in different culture media varied from 46.3 to 75.7 and are shown in Table 3. The lightness L* may vary from zero (black) to 100 (diffuse white), with lower values corresponding to darker colors. The response surfaces of L* as function of zinc, ammonium and MSG contents are shown in Figures 5a and 5b. Figure 5a shows the surface of L* as a function of MSG and Zn contents in culture media. The non-linear (quadratic) effect of Zn on the lightness of pigment can be clearly observed, with lowest lightness values for intermediate Zn concentrations. This result indicates that intermediary amount of $ZnSO_4$ added to the media may impart a darker red color to the *Monascus* pigments. However, the Zn effects shown in Figures 5a and 5b reveal similar trends, while both MSG and ammonium did not seem to cause any change in lightness. The ANOVA on L* data, shown in Table 4, demonstrate that the square of the Zn concentration influenced L* at a 5% significance level. On the contrary, the MSG and ammonium levels did not have any significant influences on L*. The equation resulting from multiple regression fitting, considering only the significant factors and obtained with R^2 = 0.812 is given by Equation 6.

$$L^* = 62.14 + 7.95\left(\frac{Zn - 0.4}{0.4}\right) \times \left(\frac{Zn - 0.4}{0.4}\right) \qquad [6]$$

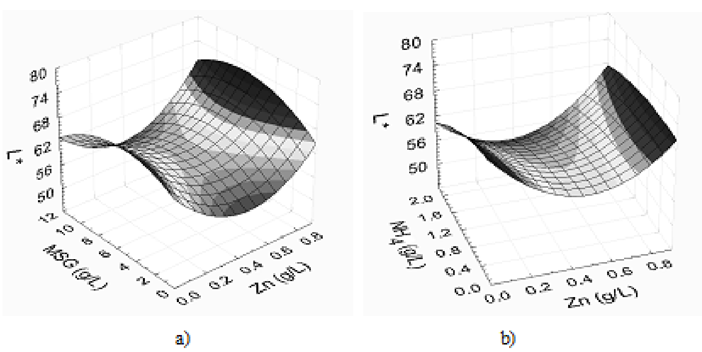


Figure 5 - Response surface of L* as a function of: a) MSG and Zn; b) NH_4 and Zn.

Color space systems, such as Hunter CIELAB, are commonly applied to assess color properties related to visual responses. In our study, the *L*, *a* and *b* followed by asterisks (*) belongs to a tridimensional CIELAB system that is different from Hunter's. *L**, *a**, and *b** relationships are nonlinear and simulate response of the eye. As a consequence, any perceptual color differences are correlated to the Euclidean distance between two colors in the *L*a*b** space. Furthermore, characteristics of extracts, as shown in Table 3, prove that higher pigment production together with best color quality (P_T = 3.9, $A_{R/Y}$ = 0.88, a*/b* = 1.29 and L = 46.3) was achieved in experiment 11, with intermediary Zn and higher NH_4 levels.

For industrial purposes, it is important to increase the overall pigment production as well as to selectively increase the proportion of red pigments. To verify the best conditions with which to obtain both high pigment production and adequate color characteristics, a Box-Behnken experimental design was applied. This factorial design approach presents the advantage of being able to show interactions between variables and reducing the number of experiments.

The culture media used was based on the nutrients employed in a large number of studies concerned with *Monascus* liquid fermentation. Commonly used media were composed of glucose, peptones, methionine, NH_4NO_3, K_2HPO_4, KH_2PO_4, $MgSO_4$, KCl, $FeSO_4$, $ZnSO_4$, $MnSO_4$, monosodium glutamate, aminoacids and dicarboxylic acids (*28, 30*), Based on a literature survey and on our preliminary experiments using several combinations of these nutrients, agitation and aeration, the culture media chosen was composed of rice flour at a concentration of 30 g/L, peptone 9 g/L, glycerin 30 g/L, glucose 110 g/L, $MgSO_4$ 1 g/L, KNO_3 2 g/L at a pH of 6.5. Both anaerobic conditions and monosodium glutamate were found to reduce the citrinin production (*22*). Ammonium stimulated the cell growth and zinc increased the red pigment production (*15*). The fermentation was performed at 37°C with moderate stirring but without aeration. Zinc sulfate, ammonium and monosodium glutamate were used as additives in varying concentrations according to the Box-Behnken experimental design.

The increase in *Monascus* pigment production in submerged culture due to low zinc content in culture media was first reported (*15*). The authors also observed an increase in pigment production in solid state fermentation with zinc addition. In both cases, the pigment production decreased above a certain value of zinc addition, and the optimum zinc concentration for submerged culture was 5 x 10^{-4} M. In our study, the weight of zinc sulfate added to the fermentation media corresponded to zero (level -1), 1.39 x 10^{-3} M (level 0) and 2.78 x 10^{-3} M (level +1). The value of zinc content for optimum pigment production in our experiments could be obtained by direct derivation of Equations 3 and 4. This derivation resulted in $ZnSO_4$ values of 0.47 g/L (1.63 x 10^{-3} M) and 0.46 g/L (1.60 x10^{-3} M) for P_R and P_T, respectively, which indicates that maximum productions of both red and total pigment could be achieved at approximately the same Zn concentration.

4. CONCLUSION

Based on the optical densities observed at 400 and 500 nm, as well as on color space characterization for all 15

© Asian Journal of Biomedical and Pharmaceutical Sciences, all rights reserved.

experimental conditions, red pigment production was increased in the substrates with intermediary concentrations of zinc and higher concentrations of ammonium. Moreover, the color quality, as indicated by L*a*b* color space measurements, was also improved by the addition of zinc. However, it is important to note the quadratic effect of zinc concentration, e.g., excessive amounts of this mineral may cause a decrease in both pigment production and color quality. This result is quite important because the quadratic effect of zinc was not reported before and it demonstrates the feasibility of high pigment production in submerged culture. In the work reported here, the red pigment production achieved was close to values reported in the literature for solid-state culture. The high costs and the difficulties of obtaining pure pigments in solid cultures makes the submerged culture an alternative strategy to improve industrial scale production of *Monascus* pigments.

5. ACKNOWLEDGEMENTS

The authors are grateful for the financial support for this research provided by the Brazilian funding agencies Fapesp (07/03048-3) and CNPq (PQ-2).

6. REFERENCES

1. I. El-Kady, S. El-Maraghy, A.N. Zohri, Mycotoxin producing potential of some isolates of *Aspergillus flavus* and *Eurotium* groups from meat products, *Microbiol. Res. 149* (1994) 297-307.
2. K.A. Scudamore, M.T. Hetmanski, Natural occurrence of mycotoxins and mycotoxigenic fungi in cereals in the United Kingdom, *Food addit contam. A, 12* (1995) 377-382.
3. M.E. Krejci, N.S. Bretz, D.A. Koechel, Citrinin produces acute adverse changes in renal function and ultra structure in pentobarbital-anesthetized dogs without concomitant reductions in [potassium] plasma, *Toxicology, 106* (1996) 167-177.
4. L. Dufossé, P. Galaup, A. Yaron, S.M. Arad, P. Blanc, K.N.C. Murthy, G.A. Ravishankar, Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality?, *Trends Food Sci. Tech. 16* (2005) 389–406.
5. G. Jacobsen, J. Wasileski, Production of Food Colorants by Fermentation, In: *Bioprocess Production of Flavor, Fragance and Color Ingredients,* A. Gabelman (Ed), John Wiley & Sons Inc., N. York, USA (1994) 205-237.
6. S. Babitha, C.R. Soccol., A. Pandey, Solid-state fermentation for the production os Monascus pigments from jackfruit seed, *Bioresource Technol. 98* (2007) 1554-1560.
7. P.J. Blanc, J.P. Laussac, J. Le Bars, M.O. Loret, A. Pareilleux, D. Prome, A.L. Santerre, G. Goma, Characterization of monascidin A from Monascus as citrinin, *Int. J. Food Microbiol. 27* (1995) 201-213.
8. E.Z. Panagou, P.N. Skandamis, G.J. Nychas, Modelling the combined effect of temperature, pH and aw on the growth rate of *Monascus ruber*, a heat-resistant fungus isolated from green table olives, *J. Appl. Microbiol. 94* (2003) 146-156.
9. L. Pastrana, P.J. Blanc, A.L. Santerre, M.O. Loret, G. Goma, Production of red pigments by *Monascus ruber* in synthetic media with a strictly controlled nitrogen source, *Process Biochem. 30* (1995) 333-341.
10. N. Lotong, P. Suwanarit, Fermentation of ang-kak in plastic bags and regulation of pigmentation by initial moisture content**,** *J. Appl. Bacteriol. 68* (1989) 565-570.
11. Y.H. Sung: Yeasts in bT'ien-Kung K'ai-WuQ. In: *Chinese Technology in the Seventeenth Century*, E.T. Sun & Z. Sun (Eds.), Pennsylvania State University Press, University Park, PA (1966) pp. 292– 294.
12. J.Q. Hu, Production and application of red fermentation rice. In: *Light Industry Press*, Beijing, China (1997) pp. 1 – 40 (in Chinese).
13. F. Chen, X. Hu, Study on red fermented rice with high concentration of monacolin K and low concentration of citrinin, *Int J. Food Microbiol. 103* (2005) 331-337.
14. H.C. Wong, Y.S. Bau, Pigmentation and antibacterial activity of fast neutron-and X-ray-induced strains of *Monascus purpureus* Went, *Plant Physiol. 60* (1977) 578-581,
15. Y.S. Bau, H.C. Wong, Zinc effects on growth, pigmentation and antibacterial activity of *Monascus purpureous*, *Physiol. Plantarum, 46* (1979) 63-67.
16. J. Fink-Gremmels, J. Dresel, L. Leistner, Use of Monascus extracts as an alternative to nitrite in meat products. *Fleischwirtschaft, 71* (1991) 1184-1186.
17. L. Martinkova, P. Patakova-Juslova, V. Krent, Biological activities of oligoketide pigments of *Monascus purpureus*. *Food Addit. Contam. A, 16* (1999) 15-24.
18. M. Kuba, K. Tanaka, M. Sesoko, F. Inoue, M. Yasuda, Angiotensin I-converting enzyme inhibitory peptides in red-mold rice made by *Monascus purpureus*. *Process Biochem. 44* (2009) 1139-1143.
19. H.C. Wong, P.E. Koehler, Production and isolation of an antibiotic from *Monascus purpureus* and its relationship to pigment production. *J. Food Sci. 46* (1981) 589-592.
20. C.E. Fabre, G. Goma, P.J. Blanc, Production and food applications of the red pigment material. *Book of Abstracts of the Symposia on Monascus Cultures and Applications*, Institut Nacionale des Sciences Apliqués, Toulouse, France (1998) July 8-10.
21. H.C. Wong, P.E. Koehler, Production of red water-soluble Monascus pigments. *J. Food Sci. 48* (1983) 1200-1203.
22. H. Hajjaj, P. Blanc, E. Groussac, J.L. Uribelarrea, G. Goma, P. Loubiere, Kinetic analysis of red pigment and citrinin production by *Monascus ruber* as a function of organic acid accumulation. *Enzyme Microb. Tech. 27* (2000) 619-625.
23. M.R. Johns, D.M. Stuart, Production of pigments by *Monascus* purpureus in solid culture. *J. Ind. Microbiol. 8* (1991) 23-38.
24. A.M. Moharram, M.M. Emam, M.A. Ismail. Chemical profile of Monascus ruber strains. *Food Technol. Biotechnol.* (2012) In press. Available at: http://www.ftb.com.hr/7.Moharram_Et_al.pdf
25. Y.P. Chen, C.P. Tseng, I.L. Chien, W.Y. Wang, L.L. Liaw, G.F. Yuan. Exploring the distribution of citrinin biosynthesis related genes among Monascus species. *J. Agr. Food Chem. 56* (2008) 11767-11772.
26. S.S. Teng, W. Fedheim, Anka and anka pigment production. *J. Ind. Microbiol. Biot. 26* (2001) 280-282.
27. J.H. Kim, H.J. Kim, J.H. Oh, C.S. Shin, Morphology control of Monascus cells and scale-up of pigment fermentation. *Process Biochem. 38* (2002) 649-655.
28. E.I. Pisareva, A.V. Kujumdzieva, Influence of Carbon and Nitrogen Sources on Growth and Pigment Production by *Monascus pilosus* C_1 Strain. *Biotechnol. Biotec. Eq. 24* (2010) 501-506.
29. M. Box, W.G. Hunter, J.S. Hunter, *Statistics for experimenters*, John Wiley & Sons, New York, USA (1978).
30. P. Pattanagul, R. Pinthong, A. Phianmongkhol, S. Tharatha, Mevinolin, Citrinin and Pigments of Addlay Angkak Fermented by *Monascus* sp. *Int. J. Food Microbiol. 126* (2008) 2-23..

Conflict of Interest: None Declared

© Asian Journal of Biomedical and Pharmaceutical Sciences, all rights reserved.