Research article

Cheese whey as a potential substrate for *Monascus* pigments production

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Abstract: The present study had the objective of producing pigments by *Monascus ruber* in solid medium, using the cheese whey as substrate. Different cheese whey concentrations (0, 5, 10, 20, 40 and 80 g L^{-1}) and pH values (2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0) were tested. There was growth of the microorganism for all concentrations of cheese whey applied and there was a significant difference between the radial growth rates of the fungus obtained for each medium when compared to the control. When analyzed under different pH values, *M. ruber* growth rates were higher at pH 6.0 in culture medium containing 20 g L^{-1} of whey powder. At extreme pH values, such as pH 2.0 and 8.0, the growth of the fungus presented inhibition and the visual aspect differed, presenting yellow and red coloration, respectively. The growth rate at pH 3.0, 4.0 and 5.0 did not differ significantly ($p > 0.05$), however, it was observed that in different pH values, orange and red pigments were produced. These results demonstrate the potential of cheese whey as a substrate for *Monascus* growth and, consequently, the production of pigments.

Keywords: biopigments; solid culture; radial growth rate; cheese whey; agro-industrial residues; *Monascus* pigments; biotechnological applications

1. Introduction

Monascus is a filamentous fungus widely used in Asian countries to produce a red dye known as "Anka", widely used as a food ingredient in typical foods. Species of this genus are widely used for coloring and improving the appearance of food [1].

Currently, *Monascus* pigment production is already useful for coloring meat products. The interest in these red pigments by the meat processing industry has increased due to the fact that the substances traditionally used (nitrite or nitrate salts) have known carcinogenic and teratogenic effect in the human organism [2]. *Monascus* fermented products have a number of functional secondary

metabolites, including anti-inflammatory pigments (such as monascin and ankaflavine), monacolines, dimerumic acid, and γ-aminobutyric acid [3]. Several studies have demonstrated that these secondary metabolites have anti-inflammatory, anti-oxidant, and anti-tumor activity [4–6].

Monascus sp. can produce at least six important related pigments, which can be divided into three groups: two are orange (rubropunctatin and monascorubrine), two are yellow (monascin and ankaflavine) and two are red (rubropunctamine and monascorubramine) [7]. The orange pigment is the precursor of the other two pigments. The oxidation of the orange pigment gives rise to the yellow pigment and the reaction of that with nitrogenous compounds produces red pigments. These pigments remain intracellular because of their high hydrophobicity and are only excreted in the medium after reacting with proteins or amino acids [8–10].

Several factors affect the production of pigments, such as temperature, oxygen presence, humidity, light intensity, pH and the composition of the medium (carbon and nitrogen sources) [7,11–14]). The most commonly used carbon sources as a substrate for *Monascus* growth are glucose, sucralose and starch. The highest growth is usually observed in glucose. However, other sources of carbon have been used as a substrate for the growth of *Monascus*, mainly residues and by-products from the food industry, such as rice, wheat flour, corn cob, jackfruit powder, glycerol and malt [12,15–17].

Cheese whey represents an agro-industrial residue with high organic matter content (lactose, protein and fats), however, only 57% of the world total of cheese whey is reused industrially. The disposal of this by-product residue, in addition to environmental pollution, is a waste of protein material, since it retains about 55% of milk nutrients [18]. Therefore, besides being used as a by-product in the production of industrialized foods and beverages [19,20] and protein supplement, cheese whey has also been widely used as a substrate in biotechnological application, such as the generation of ethanol, aroma compounds, functional food additives and carotenoids [21]. However, there are no records of the use of cheese whey for the production of pigments by *Monascus ruber*, which evidences the originality of the present study. In a recent research, Costa and Vendruscolo [22] studied the production of pigments by *Monascus ruber* CCT 3802 using lactose as substrate in submerged fermentation. This paper suggests the use of cheese whey-a residue and also a lactose-rich by-product of the dairy industry-in the production of biopigments. Thus, this work had the objective of producing pigments by *Monascus ruber* in solid medium, using the cheese whey as substrate.

2. Material and methods

2.1. Microorganism

Monascus ruber CCT 3802 was obtained from the Tropical Culture Collection André Tosello (Campinas-SP, Brazil). The strain was frozen at −20 °C after adding 100 μL glycerol mL^{-1} spore suspension as a cryoprotector. The culture was maintained on potato dextrose agar sterilized at 121 °C for 15 min, incubated at 30 °C for 7 days and subsequently stored at 4 °C. Maintenance was performed in sloped test tubes containing potato dextrose agar, previously sterilized at 121 °C for 15 min.

2.2. Cheese whey

The cheese whey powder used in this experiment was provided by *Lactosul Indústria de Laticínios LTDA* (Piranhas, Goiás, Brazil) and it was submitted to the following analyzes: residues by

incineration, proteins, total fats, and lactose reducing sugars. Such analyzes were carried out at the Center for Food Research (Goiânia, Goiás, Brazil) to know the composition of the product to be used as a substrate. The methodologies used by the laboratory are official to control products of animal origin, according to Normative Instruction No. 30, of June 26, 2018, of the Ministry of Agriculture, Livestock and Supply, Brazil.

2.3. Culture medium

The growth and pigments production under different cheese whey concentrations and pH values were studied. The study of one variable at a time was carried out. Different concentrations of cheese whey (5, 10, 20, 40 and 80 g L^{-1}) were added in potato dextrose agar (pH 5.5), which were autoclaved at 121 °C for 15 min. Plates containing only the culture medium were also evaluated for control. The concentration of cheese whey that best showed results in the first assay was applied to evaluate the production at different pH values.

After autoclaving and as a liquid, the culture medium had pH adjusted in the range of 2.0 to 8.0 with with HCl or NaOH (10% w v^{-1}), also previously autoclaved. As a parameter for this test, the radial growth was carried out with culture medium containing lactose, adjusting to the same pH ranges evaluated for the cheese whey assay.

2.4. Inoculation and radial growth

Culture media, previously autoclaved at 121 °C for 15 min, were poured into 100 mm Petri dishes. To measure radial growth rates, we adapted the methods of Gabiatti et al. [23] and inoculated the center of each plate with 0.1 mL of spore suspension containing 0.1% (w v^{-1}) bacteriological agar. Bases of plates were divided into three segments that allowed the calculation of an average diameter in cases where growth was irregular. Plates were incubated at 28 °C and the colony diameter was measured with a scalimeter every 24 h. The radial growth rate of the colonies was obtained by the slope of the linear regression of the radius of the colonies as a function of the cultivate time, as presented in Equation 1.

$$r(t) = V_{cr}t + a \qquad (1)$$

where r is the radius of the colony (mm), V_{cr} is the radial growth rate (mm h^{-1}), t is the cultivation time (h) and a is the linear regression constant. All experiments were performed in quintuplicate and were repeated at least twice. To verify if there was a significant difference between the rates from the regression curves, the *t-test* by Statistica 7.1 software was used.

2.5. Extraction and pigment quantification

The *M. ruber* colonies were carefully scraped off the surface of the solid medium with a spatula, removing as much of it as possible, and were added in 125 mL Erlenmeyer flasks containing 35 mL of 95° GL ethyl alcohol. The samples were placed in a water bath at 40 °C for 1 h under constant stirring. The sample was submitted to the filtration on Whatman # 1 filter paper, pre-dried (60 °C for 2 h) and weighed, avoiding discharging the biomass. The filtered (intracellular extract) was submitted to scanning in spectrophotometer UV/V is Lambda 45 (Perkin Elmer, Inc, Connecticut,

USA) in the range from 320 to 550 nm. Pigments produced by *Monascus ruber* CCT 3802 were expressed in absorbance unit (AU). Based on the pigment spectrum of the *Monascus* genus, the absorbance of pigments occurs at specific wavelengths: 400, 470, and 510 nm, whose values correspond to the absorption of yellow, orange and red pigments, respectively [24]. These wavelengths were used for analysis in this study.

2.6. Biomass

In the flasks, still containing the biomass and culture medium, 50 mL of distilled water were added and autoclaved at 121 °C for 5 min for complete dissolution of the potato dextrose agar. The biomass was quantified gravimetrically, by performing vacuum filtration with the same Whatman # 1 filter paper previously dried and weighed. The retained material was oven dried at 85 °C for 24 h. The set was cooled in a desiccator for 15 min and weighed in analytical balance [25].

2.7. Color characteristics

The intracellular extract was also analyzed for color characteristics. *L**, *a** and *b** values were measured by total transmittance in colorimeter ColorQuest® XE, HunterLab, (Hunter Associates Laboratory, Inc., Virginia, USA) with the CIELAB color system. Those values were used to calculate *chroma* (*C**) and *Hue* angle (*h_{ab}*) values, as presented in Equation 2 and 3 [26].

$$Chroma = [(a^*)^2 + (b^*)^2]^{1/2} \tag{2}$$

$$h_{ab} = tan^{-1}\left(b^*/a^*\right) \tag{3}$$

2.8. Statistical analyses

The data were submitted to a univariate analysis of variance (ANOVA) and the means obtained for each treatment submitted to Tukey's test using Statistica version 5.0 software at a 5% level of significance.

3. Results and discussion

The cheese whey analysis is presented on Table 1. Those results show that the cheese whey contains almost 32% of lactose as reducing sugar.

Table 1. Physico-chemical analysis of cheese whey used as a substrate for *Monascus ruber* CCT 3802 pigment production.

Component	Composition
Lactose [g (100 g)$^{-1}$]	32.27
Total fats [g (100 g)$^{-1}$]	1.49
Total proteins [g (100 g)$^{-1}$]	10.59
Fixed mineral residue [g (100 g)$^{-1}$]	6.30

Figure 1 shows the radial growth curves of *M. ruber* under different concentrations of cheese whey in the culture medium. The *lag* phase in this study was 48 h for all the colonies, independent of the culture medium studied, because the curve of all showed a slow growth low from time 0 to 48 h, demonstrating adaptation to the supplied substrate. Based on the mean values of the radius of the colonies of *M. ruber*, measured daily for each culture medium, and discarding the lag phase, the regression equations with the respective correlations and radial growth rates for *Monascus ruber* were determined, as shown in Table 2. The *M. ruber* colonies growth was observed for all the cheese whey concentrations and there was significative difference between the radial growth rates ($p \leq 0.05$) when they are compared with the control. *M. ruber* radial growth rate ranged from 0.087 to 0.144 mm h^{-1}.
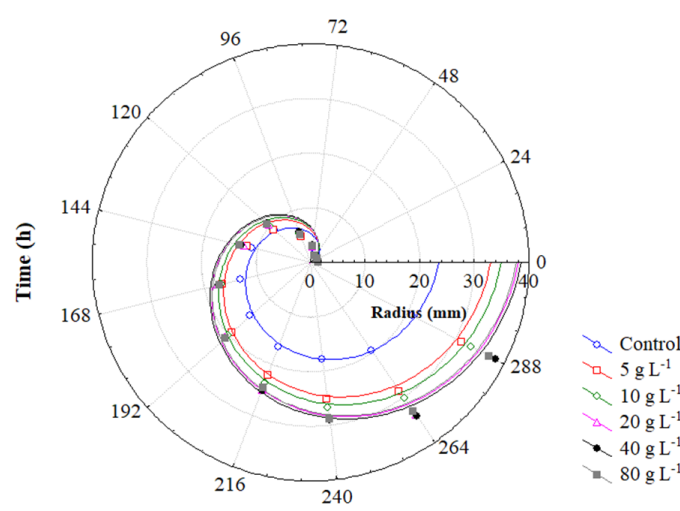
Figure 1. Radial growth curves of the *Monascus ruber* CCT 3802 colonies cultivated at different cheese whey concentrations.

Table 2. Linear regression equation, regression coefficient (R^2) and radial growth rate (V_{CR}) for *Monascus ruber* CCT 3802 colonies cultivated at different cheese whey concentrations.

Cheese whey (g L^{-1})	Regression Equation	R^2	V_{CR} (mm h^{-1})
0	$r(t) = 0.087 \times t + 1.4$	0.980	0.087 ± 0.003[d]
5	$r(t) = 0.123 \times t + 1.4$	0.990	0.123 ± 0.010[c]
10	$r(t) = 0.131 \times t + 1.4$	0.993	0.131 ± 0.013[bc]
20	$r(t) = 0.142 \times t + 1.4$	0.982	0.142 ± 0.004[ab]
40	$r(t) = 0.144 \times t + 1.4$	0.989	0.144 ± 0.003[a]
80	$r(t) = 0.141 \times t + 1.4$	0.986	0.141 ± 0.006[ab]

[a-d] Means followed by the same letter in the column do not differ significantly from each other, by the Tukey test, at the 5% level of significance.

Although the highest radial growth rate had been to 40 g L^{-1} cheese whey concentration (0.144 mm h^{-1}), this growth was not significantly different from the gotten rate in 20 g L^{-1} added (0.142 mm h^{-1}), but it was significative different ($p \leq 0.05$) from 10 g L^{-1} concentration, which represents 10% increased of *M. ruber* growth. Because of this finding, the concentration of 20 g L^{-1}

was used for the analysis regarding the pH variation. Furthermore, there was not significative difference in *Monascus* growth when the 40 and 80 g L^{-1} concentrations were compared.

The scanning analysis presented in Figure 2, is directly proportional to the dry biomass obtained and evidences the influence of the cheese whey concentration in the production of pigments. In all the spectra there is the peak in 400nm, demonstrating the presence of the yellow pigment. The wavelengths, at 510 nm, demonstrate the presence of red pigment. It is known in the present scientific environment that it is not possible to produce a single pigment during the *Monascus* fermentation. However, the pigment composition may be shaped to the conditions and composition of the medium so that blends of yellow, orange and red pigments are obtained. The absorbance for the peaks in 400 nm (absorbing purple color) and in 510 nm (absorbing green color) were higher to the samples derived from the 80 g L^{-1} of cheese whey concentration, that showed red intense color reflected.
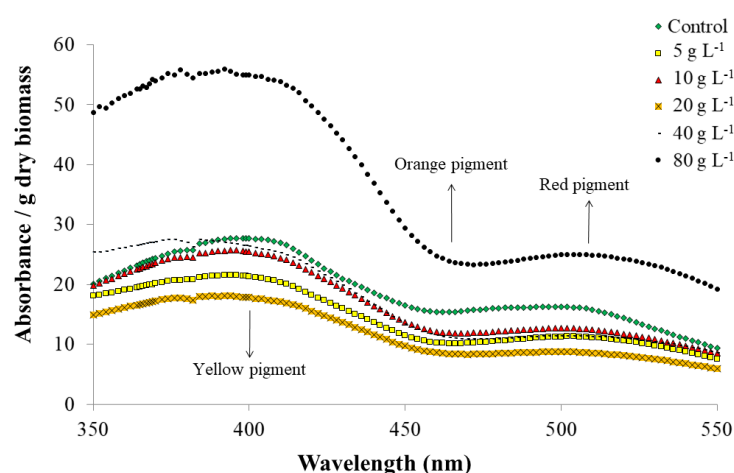

Figure 2. Scanning spectrometer analysis of the pigments produced by *Monascus ruber* CCT 3802 colonies cultivated at different cheese whey concentrations.

Table 3 shows the color characteristics evaluated in a colorimeter with the CIELAB color system of the pigments produced by *Monascus ruber* CCT 3802 when growing at different cheese whey concentrations. All values of *L**, *a** and *b** were positive for all samples, indicating that, when plotted in a polar coordinate system, all values are available in the first quadrant, which means they refer to colors close to red and yellow. The *h*$_{ab}$ values ranged from 48 to 56, indicating that the color ranged from deep red to dark orange. Pigment samples derived from the culture medium containing 5 and 10 g L^{-1} of cheese whey were slightly darker and reddish than the control sample. On the other hand, the sample derived from 20 g L^{-1} of cheese whey concentration presented a lighter color, less red and more yellow than the control, by the way, it is visible the absorbance peak in 400 nm and also in 510 nm are lower than the other samples. Figure 3 shows the appearance of colonies of *M. ruber* under different cheese whey concentrations. The colonies were well developed, demonstrating the diffusion halo and flocculent appearance of aerial mycelium, besides the characteristic production of pigments. *M. ruber* colonies presented typical characteristics: planar and rounded colonies, with aerial development, initially white, turning to reddish-brown color throughout the incubation time, with diffusion of the pigments through the agar [27].

Table 3. Colorimetric analysis of CIELAB system of the pigments produced by *Monascus ruber* CCT 3802 cultivated at different cheese whey concentrations.

Cheese whey (g L^{-1})	CIELAB System					Color[1]
	L^*	a^*	b^*	C^*	h_{ab} (°)	
0	76.40 ± 4.83[b]	38.38 ± 6.80[b]	40.00 ± 6.56[bc]	55.43	48.18	Intense orange
5	69.27 ± 3.67[c]	49.15 ± 4.61[a]	58.36 ± 6.80[a]	76.30	49.90	Orange/red
10	72.41 ± 2.08[bc]	44.89 ± 2.95[ab]	53.94 ± 4.57[a]	70.18	50.24	Orange/red
20	77.99 ± 2.87[b]	36.68 ± 4.65[b]	49.16 ± 2.63[ab]	61.34	53.27	Red
40	86.36 ± 1.76[a]	23.19 ± 2.90[c]	34.71 ± 3.69[c]	41.75	56.26	Intense red
80	76.79 ± 3.39[b]	38.44 ± 5.09[b]	52.68 ± 6.55[a]	65.21	53.89	Intense red

[1]Color interpretation based on visual analysis. [a-c] Means followed by the same letter in the column do not differ significantly from each other, by the Tukey test, at the 5% level of significance.
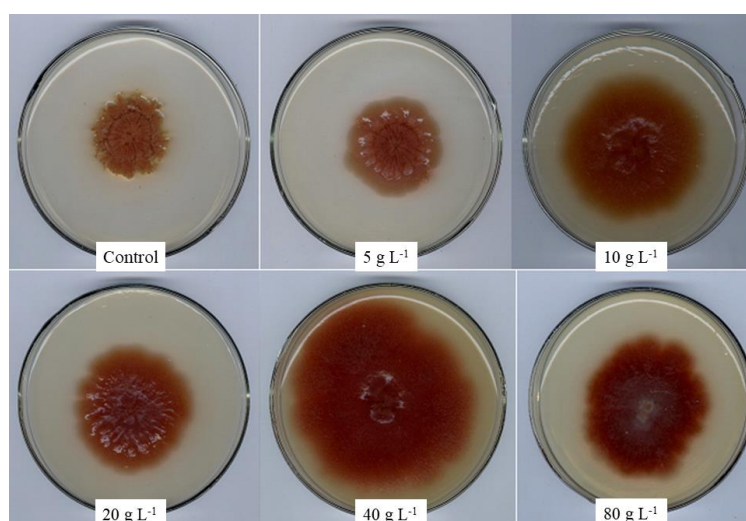
Figure 3. Visual aspect of *Monascus ruber* CCT 3802 colonies cultivated at different cheese whey concentrations (336 h).

Pisareva and Kujumdzieva [28] studied the influence of the carbon and nitrogen sources on the production of pigments by *Monascus pilosus*. Sixteen carbon sources were analyzed, including lactose, the source of carbon present in the substrate analyzed in this study. According to the results, there was no production of pigments and biomass in the presence of lactose. The nitrogen sources studied influenced the production of pigments by the strain. However, Costa and Vendruscolo [22] analyzed the production of pigments in the presence of lactose and found the production of pigments, although lower than the production of pigments in the presence of glucose. Vendruscolo et al. [24] studied the influence of the amination of the orange pigment, evidencing that the source of nitrogen can affect the coloration of the orange pigment to red. The orange pigment is the first product of biosynthesis and the precursor of the other pigments [8,10,29,30]. Therefore, not only lactose, but also the presence of proteins and other minerals present in the cheese whey favoured nutritional enrichment of the culture medium, providing conditions favourable to the growth and production of pigments by *M. ruber*.

The effect of different pH values on the production of pigments by *M. ruber* was investigated. The regression equations and correlation coefficient from the slopes of the regressions (Table 4)

showed that the growth of the colonies of *M. ruber* presented a linear behaviour. It was observed that the highest growth rate was obtained at pH 6.0 (0.134 ± 0.008 mm h^{-1}), and the lowest at pH 8.0 (0.019 ± 0.010 mm h^{-1}), both in culture containing 20 g L^{-1} of cheese whey. However, it is notorious that the amount of red pigments produced at pH 6.0 exceeds production at pH 8.0, demonstrates that the simple adjusted of the pH can increase in approximately 7 times the production of biopigments.

The colonies growing at pH 6.0 in medium containing lactose showed 45% of the average growth rates of colonies in media containing cheese whey, demonstrating the potential of this by-product of the dairy industry to produce microbial pigments. The highest radial growth rate of *M. ruber* in culture medium containing 10 g L^{-1} of lactose corresponded to a growth of 0.072 mm h^{-1} (pH 5.0).

Table 4. Linear regression equation, regression coefficient (R^2) and radial growth rate (V_{CR}) for *Monascus ruber* CCT 3802 colonies cultivated at different pH values.

pH	Lactose			
	Regression equation	R^2	V_{CR} (mm h^{-1})	Color[1]
2.0	$r(t) = 0.037 \times t + 1.4$	0.951	0.037 ± 0.003^d	Yellow
3.0	$r(t) = 0.055 \times t + 1.4$	0.969	0.055 ± 0.002^{bc}	Orange
4.0	$r(t) = 0.070 \times t + 1.4$	0.976	0.070 ± 0.003^a	Orange
5.0	$r(t) = 0.072 \times t + 1.4$	0.983	0.072 ± 0.002^a	Red
6.0	$r(t) = 0.060 \times t + 1.4$	0.949	0.060 ± 0.003^b	Red
7.0	$r(t) = 0.054 \times t + 1.4$	0.919	0.054 ± 0.007^{bc}	Red
8.0	$r(t) = 0.049 \times t + 1.4$	0.860	0.049 ± 0.002^c	Red
	Cheese whey (20 g L^{-1})			
2.0	$r(t) = 0.098 \times t + 1.4$	0.956	0.098 ± 0.007^c	Yellow
3.0	$r(t) = 0.113 \times t + 1.4$	0.988	0.113 ± 0.009^b	Orange
4.0	$r(t) = 0.113 \times t + 1.4$	0.981	0.113 ± 0.005^b	Orange
5.0	$r(t) = 0.127 \times t + 1.4$	0.987	0.127 ± 0.004^{ab}	Red
6.0	$r(t) = 0.134 \times t + 1.4$	0.977	0.134 ± 0.007^a	Red
7.0	$r(t) = 0.067 \times t + 1.4$	0.768	0.067 ± 0.004^d	Red
8.0	$r(t) = 0.019 \times t + 1.4$	0.822	0.019 ± 0.010^e	Red

[1] Color interpretation based on visual analysis. [a-e] Means followed by the same letter in the column do not differ significantly from each other, by the Tukey test, at the 5% level of significance.

Figure 4 shows the evolution of the radial growth of the colonies of *M. ruber* at different pH values. The high growth of the fungus in medium containing cheese whey comparing to the culture medium containing just lactose can be explained by the cheese whey composition (Table 1), which, although providing 3.6 g of lactose less, is rich in nutrients, such as proteins and fats, as well as lactose, which provide the necessary energy for the synthesis of biopigments for a longer period, since when the carbon source ceases the microorganism will tend to perform its metabolic pathway from other available nutrients in the middle for its maintenance.
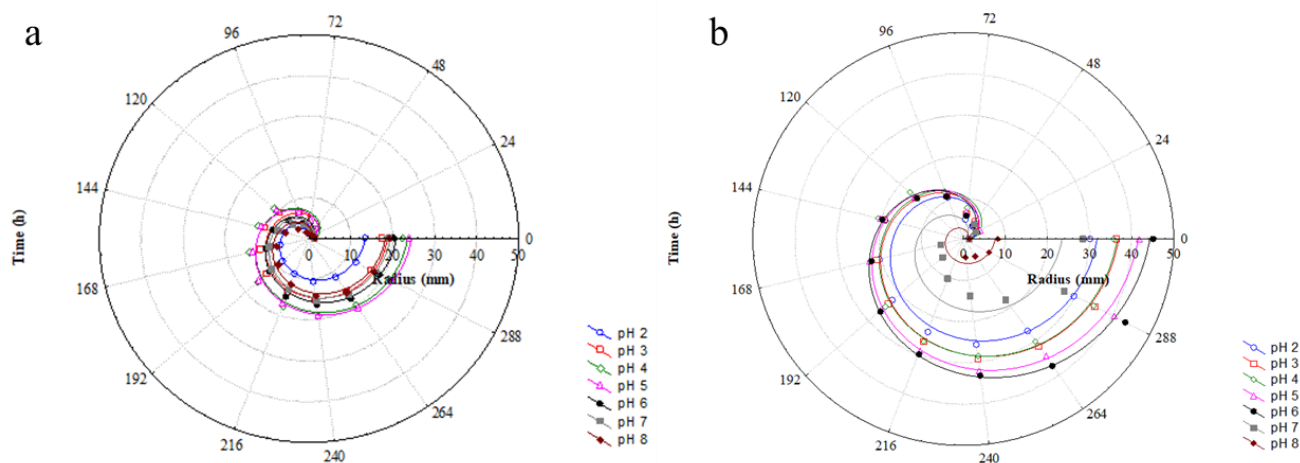
Figure 4. Radial growth curves of the *Monascus ruber* CCT 3802 colonies cultivated at different pH values (a) 10 g L^{-1} of lactose and (b) 20 g L^{-1} of cheese whey.

About the scanning spectra (Figure 5), the culture medium contains 10 g L^{-1} of lactose, at pH 2.0, it is possible to identify the absence of a peak at 510 nm, demonstrating the predominance of the yellow pigment, which does not occur in the pigment extracted from the culture medium containing cheese whey. It is observed that in the presence of cheese whey red pigment production was higher at pH 3.0, 4.0 and 5.0. The scanning spectra for the pigments analyzed from the media at pH 6.0 and 7.0 were similar. At pH 8.0, the predominance of the red pigments is evidenced in the medium containing lactose. This is since the colony in this culture medium has obtained greater radial growth, which, consequently, allowed a greater extraction of pigments.

Table 5 presents the positive values for *L**, *a** and *b**, that correspond to the first quadrant colors at CIELAB System, between red and yellow. It can be noted that the pigments produced by *M. ruber* at pH 2.0 presented higher values for *b**, which shows the predominance of the yellow pigment. However, in the same pH, for culture medium containing cheese whey, the highest value of *a** is evident, which means the presence of red color more pronounced than in lactose. This can also be evidenced by the higher value of the h_{ab}. At pH 3.0 and 4.0, for both media, the samples showed h_{ab} between 46.47° and 39.17°, respectively, indicating colors between red and yellow. Values close to 0 for h_{ab} indicate the red color, which can be observed for the pigments extracted in culture media with pH above 5.

Table 5. Colorimetric analysis of CIELAB system of the pigments produced by *Monascus ruber* CCT 3802 cultivated at different pH values.

pH	Lactose				
	*L**	*a**	*b**	*C**	h_{ab} (°)
2.0	78.08 ± 5.04[a]	20.79 ± 8.90[b]	99.86 ± 10.53[a]	102.21	78.24
3.0	44.61 ± 2.00[b]	62.97 ± 1.27[a]	66.28 ± 2.85[b]	91.46	46.47
4.0	53.18 ± 6.90[b]	55.32 ± 7.53[a]	45.06 ± 8.15[c]	71.37	39.17
5.0	50.06 ± 4.12[b]	58.91 ± 2.69[a]	49.05 ± 4.77[bc]	76.68	39.78
6.0	51.83 ± 3.18[b]	57.54 ± 2.87[a]	44.01 ± 4.43[c]	72.46	37.41

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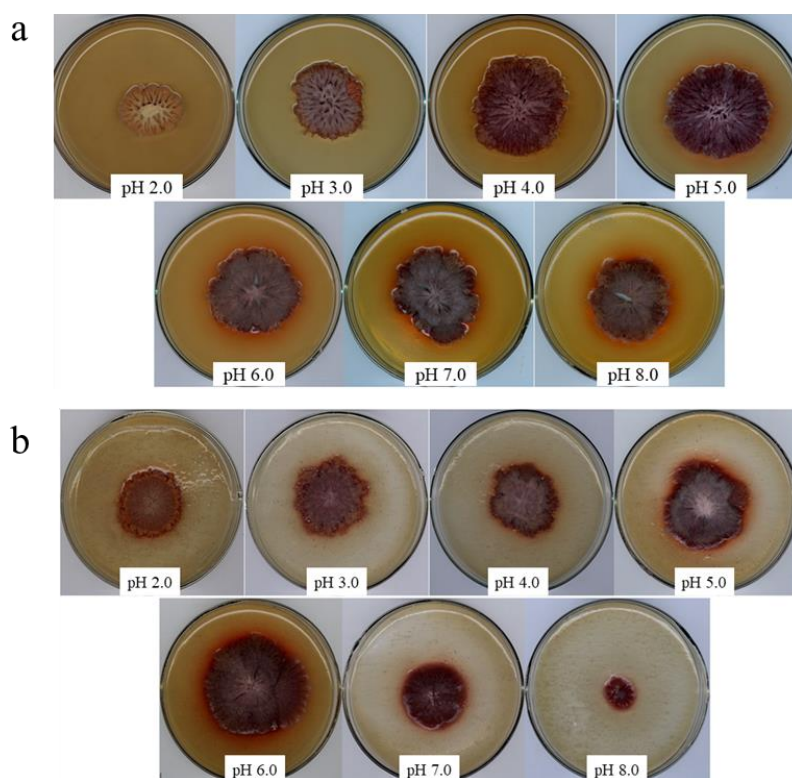| pH	Lactose				
	L^*	a^*	b^*	C^*	h_{ab} (°)
7.0	46.86 ± 3.74[b]	60.90 ± 2.27[a]	52.05 ± 6.20[bc]	80.18	40.52
8.0	44.98 ± 2.91[b]	62.12 ± 1.83[a]	54.39 ± 4.26[bc]	82.59	41.21
	Cheese whey				
2.0	60.21 ± 4.04[c]	48.79 ± 5.02[b]	82.59 ± 1.26[a]	96.02	59.44
3.0	53.55 ± 3.15[cd]	55.83 ± 3.28[ab]	51.33 ± 35.32[bc]	75.92	45.59
4.0	47.89 ± 2.96[d]	60.68 ± 2.06[a]	54.53 ± 6.03[b]	81.64	41.94
5.0	57.51 ± 5.13[cd]	51.20 ± 5.57[ab]	40.32 ± 5.63[c]	65.18	38.22
6.0	54.53 ± 4.39[cd]	54.90 ± 4.67[ab]	43.15 ± 8.71[bc]	69.97	38.17
7.0	77.93 ± 4.17[b]	25.52 ± 5.12[c]	18.34 ± 3.29[d]	31.44	35.70
8.0	97.38 ± 2.31[a]	2.91 ± 2.69[d]	1.87 ± 1.59[e]	3.46	32.73

[a-c] Means followed by the same letter in the column do not differ significantly from each other, by the Tukey test, at the 5% level of significance.
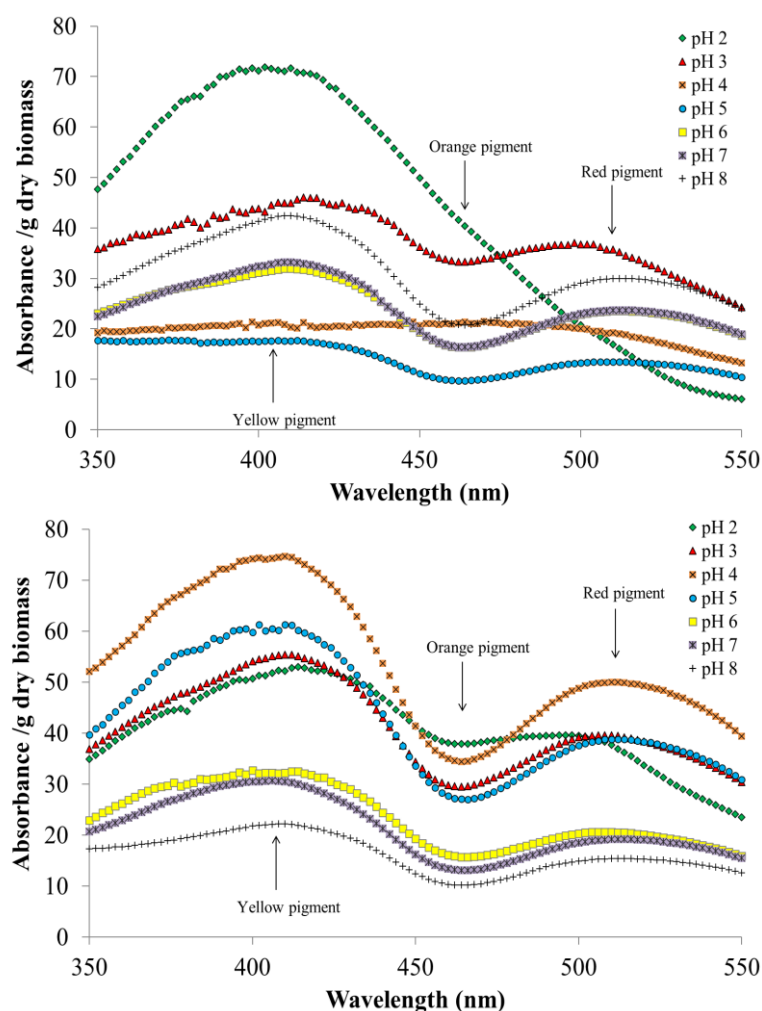
Figure 5. Scanning spectrometer analysis of the pigments produced by *Monascus ruber* CCT 3802 colonies cultivated at different pH values (a) 10 g L^{-1} of lactose and (b) 20 g L^{-1} of Cheese whey.

The visual appearance of *M. ruber* colonies can be observed on Figure 6. Colonies cultivated at pH 5.0 show more intense red color and, at pH 4.0, dark orange color. Colonies cultivated at pH 2.0 and 3.0 had inhibition of their growth, due to the extreme pH at which they were submitted. The yellow and orange color is evident for the respective pH values 2.0 and 3.0, regardless of the culture medium evaluated. However, the radial growth rate of the colony grown at pH 2.0 in the medium containing cheese whey was approximately 3 times higher when compared to the medium containing lactose alone; for the medium at pH 3.0, the rate presented was 2 times higher. Vendruscolo et al. [24] evaluated the radial growth of the colonies and the pigments produced by *M. ruber* CCT 3802 in potato dextrose agar under different pH values and concluded that pH directly influences the production of biopigments. At low pH values (2.0 to 3.0) yellow pigments were obtained, while orange pigment production was more evident at pH variations between 3.0 and 4.0. Red pigments were obtained when the fungus was submitted to pH conditions above 5.0, which also occurred in the present study.

Due to affinity for amine groups, *Monascus* pigments are often associated with proteins or cell walls, forming complex, difficult to extract pigments [30]. Therefore, a culture medium rich in nitrogen and oxygen sources is determinant in the production of red pigments, which are considered the most important pigments produced by *Monascus* since they can be used as ingredients in foods [7,31].

Figure 6. Visual appearance of the *Monascus ruber* CCT 3802 colonies cultivated under different pH values (a) media containing 10 g L^{-1} of lactose and (b) media containing 20 g L^{-1} of cheese whey.

4. Conclusions

The cheese whey presented as a potential material to the production of pigments by *Monascus ruber*. There was growth of the microorganism for all concentrations of whey applied and there was a significant difference between the growth rates of the fungus obtained for each medium when compared to the control. These results demonstrate the potential of whey as a substrate for fungus growth and, consequently, the production of pigments. In addition, there are no records of the use of whey for the production of pigments by *Monascus ruber*, which evidences the originality of the present study. Future studies should be carried out in order to demonstrate the maximization of the production of pigments from the whey as a substrate, which proved to be a cheap and profitable solution to this type of biotechnology.

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Author contribution statement

Jaqueline P. V. da Costa: Performed the experiments, analyzed and interpreted the data. Wrote the paper.
Camila F. D. Oliveira: Performed the experiments, analyzed and interpreted the data.
Francielo Vendruscolo: Conceived and designed the experiments; Contributed with the reagents, material and analytical tools. Supervision. Wrote and submitted the paper.

Conflicts of interest

All authors declare no conflict of interest in this paper.

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