Intl. J. Food . Ferment. Technol. 5(1), 47-52, June, 2015

DOI Number: 10.5958/2277-9396.2015.00006.9

RESEARCH PAPER

Physical and Chemical Stability Analysis of Thermomyces Yellow Pigment for Food Application

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Paper No. 79 Received: 14 September 2014 Accepted: 21 March 2015

Abstract

The investigation on bio-colour production for use is food through fermentation and evaluation of the yellow pigments secreted by *Thermomyces sp* in submerged culture, using sucrose and ammonium sulphate as carbon and nitrogen sources is reported. After extraction and purification, these colorants were suspended in water for evaluation. The stability of the extract was tested in different environmental conditions such as pH, heat, light, antioxidants and chemical preservatives. It has been shown that increasing the pH, temperature or exposure to light does not affect stability of the yellow pigment. The pigment extract was more stable at pH 5.1 and 8.0, temperature at 10^{O}C, 20^{O}C and 30^{O}C both in the presence and absence of light, however moderate stability was observed in antioxidants and preservatives. Thus, yellow pigment obtained from *Thermomyces* sp has a high potential to be used as a natural food colorant.

Keywords: Antioxidants, yellow pigment, light, preservatives, pH, temperature, *Thermomyces* sp

Natural colorants are considered to be safer than synthetic ones, and their applications in foods, cosmetics and pharmaceuticals are growing rapidly (Lauro, 1991). There are a number of natural pigments, but only a few are available in sufficient quantities for industrial production. Production of pigments from micro-organisms is advantageous over other sources because the avilmicroorganisms can grow rapidly which may lead to a high productivity of the product (Kim *et al.,* 1999; Joshi *et al.,* 2003, Joshi *et al.* 2011). However, replacing synthetic dyes with natural colorants offers a challenge because the colour and stability of plant pigments are dependent on several factors, including structure and concentration of the pigment, pH, temperature, light intensity, presence of, metallic ions, enzymes, oxygen, ascorbic acid, sugars and their degradation products (Velmurugan *et al.,* 2010).

The current use and the potential of using filamentous fungi as pigment and natural colorant sources for food applications are promising considering the ever rising demand by the consumers to replace their synthetic counterparts. Filamentous fungi are readily available and suitable microorganism that can be tailored to make microbial cell factories for the production of food grade pigments because of their chemical and color versatility in their pigment profile, easier large scale controlled cultivation, and a long-term history of their production of a variety of other biochemicals including biocolor (Dufossé *et al.,* 2014)

The pigments from microbial sources are a good alternative that could easily be produced in high yields and with different coloured shade pigments. Pigment producing microorganisms and microalgae are quite common in nature. Pigment produced by microorganisms includes carotenoids, melanins, flavins, quinones and more specifically monascins, violacein, phycocyanin or indigo. They have antioxidant, anti-inflammatory and anticancerous property and can better be employed as the natural source of food colorant (Mapari *et al.,* 2006 and 2010; Poorniammal *et al.,* 2011).

however, the lower stability of natural plant pigments against environmental factors could pose restriction to their utilization as food colorant in the food industry. Therefore, the present study characterize the stability of fungal pigment extracts. Our approach involved determining the effect of pH, temperature, light antioxidants and preservatives on pigment stability through time.

Materials and Methods

Growth and culture conditions

A loop full of *Thermomyces* sp from the PDA slants was inoculated into 10 ml of broth. After 2 or 3 days of growth the, inoculum was transferred to 3 Lit and 5 Lit flasks, containing the potato dextrose medium. The extracellular pigments that were excreted in the broth after 5 days of growth were harvested by filtration using Whatman No 1 filter paper. The culture extract was concentrated using vacuum rotary evaporator. The concentrated solutions were then ,lyophilized to get the dryness and stored at -20^{O}C, until they were utilized for assays.

Pigment stability to various physical and chemical conditions

pH stability

Aliquote of 5 ml of the raw yellow pigment extracts were diluted in enough water to complete 50 ml. From this solution, other solutions were prepared, with pH adjusted to several values, from 3, 5 and 9 with 0.1N NaOH or dil. HCl These solutions were incubated to 120 min. The colour intensity was read in CIELAB colorimetric system

Heat stability

For determining heat stability of the yellow pigment solution, different aliquotes were incubated at temperatures *viz*. 100, 150 and 200^{O}C for 15, 30, 60, 90 and 120 min and absorbance was read in CIELAB colorimetric system.

Light sensitivity

Pigment solution different were exposed to sunlight and fluorescent light for 15, 30 and 45 days and their light sensitivity was determined in CIELAB colorimetric system

Preservatives treatments

Aliquotes of 5 ml of the raw pigment extracts were diluted in enough water to complete 50 ml and 10 mg of preservatives sodium metabisulfite preservative and citric acid (Acidulent) were added. These solutions were incubated to 120 min. The colour intensity was read in CIELAB colorimetric system

Antioxidant treatments

Aliquotes 5 ml of the raw pigment extracts were diluted in enough water to complete 50 ml and 10 mg of antioxidants ascorbic acid and butylated hydroxyl anisole (BHA) were added. These solutions were incubated to 120 min. The colour intensity was simlarly read in CIELAB colorimetric system

Results and Discussion

Stability of fungal pigments

Stability of natural colourants towards formulations, different processing conditions, additives, pH, uniformity in distribution in products and availability of raw materials, cost economics of preparation, *etc.*, are the major factors affecting the selection and application of natural food colours in processed food products (Rao *et al.*, 2002).

pH stability of yellow pigment

. Colour intensity of pigment solution was after ph adjustment measured using CIELAB colorimeter. after incubation from 0 min to 120 min at room temperature and the stability of the pigment was measured. This pigment solution showed its stability till 2 h at pH 3, pH 5 and pH 9. The data presented in Table 1 depicts the qualitative change in the colour over the time at different pH values. The initial a*, b*, and C* values of the control and final values were noted. At pH 5, it showed maximum stability of only 6.98 % and discolouration till 120 min (b * value 38.63 to 36.99). But at pH 3 and 9 absorbance started to decrease with increase in

incubation time period. Maximum decrease was observed at pH 3 (b * value 30.69 to 26.68) with 17.48 % discolouration in the solution. At alkali pH of 9.0 (b * value 41.93 to 40.03), 11.47% discolouartion was observed. From the observations it can conclude that this pigment solution is having maximum stability at neutral pH than under acidic conditions. The pH of the medium had a major influence on colouration of the pigment solution inducing sometimes a modification in their structure. In the present study *Thermomyces* sp extract was introduced in to solutions with different pH, greater sensitivity was observed for yellow pigments at acidic pH.

We have compared our stability results with results reported in Fabre *et al.,* (1993) for the stability of *Monascus* derivatives cultured in glutamic or amino acids. Our results agree with the glutamic acid results in showing that the pigments are more stable at pH 7.0 and 9.2 than at pH 3.0 (Wong *et al.,* 1983). Fabre *et al.,* (1993) also reported that *Monascus* extracts show a red color at acidic pH values and form crystals at an extreme alkaline pH. These results are similar to our data. Our results also agree with results in Carvalho *et al.,* (2005) on the pH stability of *Monascus* and their derivatives, such as xanthomonasin A (yellow), monascorubrin (orange), and a threonine derivative of rubropunctatin (purple-red) These derivatives were also found to be more stable in neutral and/or alkaline pH ranges than in an acidic range.

Heat stability of yellow pigment

. It was observed that colour decrease was rapid in temperature 100^{O}C for 90 -120 min (b* value 100^{O}C for 90 min 49.26 to 47.59; b * value 100^{O}C for 120 min 49.26 to 47.13) and the decrease was nearly 7%. When the temperature was found to be increased from 150 to 200 ^{O}C for 15 min, pigment discolouration was found to be 4 -5% (b * value 150^{O}C for 15 min 49.26 to 46.38 ; b * value 200^{O}C for 15 min 49.26 to 46.04)

Ferreira *et al.* (1999) studied the stability of water soluble annatto exposed to various heating conditions *viz.*, 90, 100, 120, 140^{O}C for periods up to 45 min. Colour characteristics such as lightness, yellowness and redness, and norbixin contents were determined. Yellowness increased and redness decreased with increase in temperature. *Thermomyces* sp pigment showed stability up to 150^{O}C. Kaur *et al.* (2009) reported that carotenoids produced by *Rhodotorula* also got chemically denatured on exposure to heat (70-100^{O}C) for 15 minute (51% decolouration) (Table 2) . According to Bhosale *et al.* (2003) carotenoids are the main pigment produced by *Rhodotorula. Rhodotorula* pigment also gets chemically denatured on exposure to the light, heat and oxygen

Light stability of yellow pigment

The results on light stability of the yellow pigments of *Thermomyces* sp. stored under direct light conditions at room temperature during the 45 days trial period, is, shown in Table 3. The yellow pigments when stored for 15 days about 2.7 % colour (b * value for 15 days 49.26 to 44.28) was lost. At the end of 45 days, the colour loss was about 7 % (b * value for 45 days 49.26 to 43.76). The pigment when exposed to direct sunlight for 5 h, the b * value indicated that only 7 % discolouration was found. It was inferred from the results that the pigment was more stable in fluorescent light and than sunlight (Mapari *et al.,* 2009)

Preservative stability of yellow pigment

The pigment was treated with preservatives and acidulent *viz.,* citric acid and sodium metabisulfite and colour stability was analyzed after 2 h of incubation. The yellow pigment was stable in citric acid treated samples, only 3 – 7 % colour loss was observed (b * value 49.26 to 27.74). But in the sodium metabisulfite treated sample, rapid colour loss from 13 to 18% was observed (b * value 49.26 to 31.06). It was inferred that citric acid was the most suitable preservate colour followed by sodium metabisulfite**.** Residual colouration of the yellow pigments after exposure to light for 45 days however indicated less sensitivity. The results are in agreement with reports of Fabre *et al.* (1993). Where they reported that the *of* yellow pigments are stable to light but the red pigments are sensitive. The photo stability of an orange red and yellow fungal pigment extract were studied in a soft drink model medium in a citrate buffer at low and neutral pH. The result showed that enhanced photostability of fungal extracts was observed compared to the commercially available natural colourants (Table 4).

Antioxidant stability of yellow pigment

When an antioxidant such as ascorbic acid and butylated hydroxyl anisole (BHA) (0.3 g/l) were added to the pigment solution, the discolouration of pigment solution for ascorbic acid varied from 11 to 18 % (b* value 49.26 to 45.65) and BHA from 18 to 19% after 2 h incubation (b* value 49.26 to 43.20). The yellow pigment showed moderate stability to antioxidants. Lee and Chen, (1998) studied the preservative stability of *Monascus* pigments, and noted only 10% discolouration

during the period. The red and yellow pigment obtained from *Monascus ruber* when treated with antioxidants such as ascorbic acid, the discolouration of the red pigments decreased from 70 to 50% after 5 hr. The yellow components showed a slight increase in residual colouration (Fabre *et al.,* 1993) (Table 5).

Conclusion

From the results it can be concluded that pigment extract of *Thermomyces* are highly or moderately resistant to the pH, temperature, light factors and moderately resistant to antioxidant and preservatives tested. These results thus suggest that The microbial colours have the advantage of being climate independent, do not require large area for their growth and can be produced in any quantity in shorter period, shoud be stable to the conditions nosmally encountered in the foods and this could be explosed futher.

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Table 1. Effect of different pH on stability of *Thermomyces* sp pigment, with a and b CIELAB colorimetric data

Colour values	pH 3.0 (minutes of incubation)			pH 5.0 (minutes of incubation)			pH 9.0 (minutes of incubation)			Before treatment
	0	60	120	0	60	120	0	60	120	
L*	85.57	84.69	83.45	85.78	86.17	86.15	98.72	99.44	99.27	102.76
a*	-4.28	-3.98	-1.74	-3.51	-2.95	-3.1	3.93	4.14	3.79	0.29
b*	30.69	29.61	26.67	38.63	37.94	36.99	41.93	40.78	40.03	49.26
C*	30.99	29.88	26.73	38.79	38.06	37.12	42.11	40.99	40.21	49.26
h*	66.78	66.43	66.34	68.54	68.43	68.25	68.34	64.44	67.43	69.53
% Discolouration	15.06	16.97	17.48	5.42	6.00	6.98	6.47	10.54	11.47	—

% Discolouration = (tan h after treatment)$^{-1}$ – (tan h before treatment)$^{-1}$/(tan h before treatment)$^{-1}$

L* - lightness read from 0 (black) to 100 (white), a* - (positive) red colour, (negative) green colour, b* - (positive) yellow, (negative) blue colours, C* - Purity of the colour, h* - Hue

Table 2. Effect of Heat on stability of *Thermomyces* sp pigment, with a and b CIELAB colorimetric data

Colour values	100^{O}C (minutes of incubation)				150^{O}C	200^{O}C	Before treatment
	30	60	90	120	15	15	
L*	105.21	102.92	105.32	105.52	101.56	99.74	102.76
a*	0.53	0.57	0.48	0.48	-1.33	1.84	0.29
b*	47.80	47.79	47.59	47.13	46.38	46.04	49.26
C*	47.81	47.80	47.59	47.13	46.40	46.07	49.26
h*	69.45	69.24	68.12	68.11	68.71	68.55	69.53
% Discolouration	1.5	1.6	7.6	7.7	4.48	5.34	—

% Discolouration = (tan h after treatment)$^{-1}$ – (tan h before treatment)$^{-1}$/(tan h before treatment)$^{-1}$

Table 3. Effect of light on stability of *Thermomyces* sp pigment, with a and b CIELAB colorimetric data

Colour values	Fluorescent light (days of incubation)			Sun light	Before treatment
	15	30	45	10 am to 3 pm (5 hours)	
L*	99.76	98.93	99.34	99.32	102.76
a*	-7.44	-11.37	-11.59	-6.67	0.29
b*	44.28	43.89	43.76	43.18	49.26
C*	44.90	45.34	45.27	44.69	49.26
h*	68.21	66.27	68.24	68.45	69.53
% Discolouration	2.68	6.67	7.02	7.07	—

% Discolouration = (tan h after treatment)$^{-1}$ – (tan h before treatment)$^{-1}$ / (tan h before treatment)$^{-1}$

Table 4. Effect of preservatives on stability of *Thermomyces* sp pigment, with a and b CIELAB colorimetric data

Colour values	Citric acid (minutes)			Sodium metabisulphate (minutes)			Before treatment
	0	60	120	0	60	120	
L^*	86.56	86.35	86.04	87.01	86.56	86.78	102.76
a^*	-6.03	-4.71	-4.72	-5.12	-5.09	-5.15	0.29
b^*	28.63	27.39	27.34	31.86	31.65	31.06	49.26
C^*	29.26	27.79	27.74	32.27	32.06	31.48	49.26
h^*	68.93	68.84	68.12	67.14	66.82	66.25	69.53
% Discolouration	3.31	3.79	7.66	13.07	14.81	18.0	—

% Discolouration = (tan h after treatment)$^{-1}$ – (tan h before treatment)$^{-1}$ / (tan h before treatment)$^{-1}$

Table 5. Effect of antioxidants on stability of *Thermomyces* sp pigment, with a and b CIELAB colorimetric data

Colour values	Butylated hydroxyl anisole (BHA)			Ascorbic acid			Before treatment
	0	60	120	0	60	120	
L^*	101.05	100.24	99.01	101.07	101.49	101.63	102.76
a^*	2.41	3.82	2.36	1.47	1.1	0.39	0.29
b^*	43.89	43.59	43.20	45.75	45.67	45.65	49.26
C^*	43.95	43.76	43.26	45.78	45.65	45.65	49.26
h^*	67.38	67.09	66.09	66.23	66.23	66.01	69.53
% Discolouration	11.75	13.3	18.8	18.10	18.10	19.30	—

% Discolouration = (tan h after treatment)$^{-1}$ – (tan h before treatment)$^{-1}$/(tan h before treatment)$^{-1}$

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