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Photostability of Natural Orange−Red and Yellow Fungal Pigments in Liquid Food Model Systems

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The variation in the photostability among the currently authorized natural pigments limits their application span to a certain type of food system, and more robust alternatives are being sought after to overcome this problem. In the present study, the photostability of an orange−red and a yellow fungal pigment extract produced by ascomycetous fungi belonging to the genera *Penicillium* and *Epicoccum*, respectively, were studied in a soft drink model medium and in citrate buffer at low and neutral pH. The quantitative and qualitative color change pattern of the fungal pigment extracts indicated an enhanced photostability of fungal pigment extracts compared to the commercially available natural colorants *Monascus* Red and turmeric used as controls. Yellow components of the orange−red fungal pigment extract were more photostable than the red components. Chemistry of the photodegradation of the orange−red pigment extract was studied by high-performance liquid chromatography−diode array detection−mass spectrometry (HPLC−DAD−MS), and a light-induced formation of a structural analogue of sequoiamonascin C, a *Monascus*-like polyketide pigment discovered in the extract of *Penicillium aculeatum* IBT 14263 on yeast extract sucrose (YES) medium, was confirmed in the soft drink medium at pH 7.

KEYWORDS: *Monascus* pigments; polyketide; *Epicoccum*; *Penicillium*; sequoiamonascin C

INTRODUCTION

Naturally derived colorants look set to overtake synthetic food colorants in market value because of the rising demand for the clean label ingredients. The stability of natural colorants is quite an issue because, on the one hand, the color stability is a prerequisite for successful application, while on the other hand, to absorb light and elicit a color visible to the human eye, most of the currently employed natural pigments are highly unsaturated compounds and are thus prone to light, heat, and oxygen disintegration and may change color depending upon the pH (*1*). However, there are large differences in the stability of different classes of existing natural food colorants (*2*). Vegetable carbon and caramel are very stable toward heat, light, and oxygen. Carminic acid and carmine are also quite stable but not as stable as vegetable carbon and caramel. On the other hand, turmeric is rapidly bleached by light and beet root pigments turn brownish even under mild heating conditions (*2*). In many cases, the stability issues in relation to heat, light, and pH currently limit the application span of certain natural colorants to certain types of products that "fit" the stability requirements of the colorant. Hence, there is a great scope for the discovery of relatively more stable natural pigments that may have wider industrial applications without the potential use of formulation techniques. Recently, microorganisms including ascomycetous fungi have attracted attention as additional or alternative readily available sources of naturally derived food colorants that are independent of agro-climatic conditions (*1*, *3*, *4*). Pigments produced by such a kind of fungi can be broadly classified as carotenoids and/or a structurally diverse polyketide class of pigments synthesized as secondary metabolites; the example of the latter kind are *Monascus* pigments that have been used as natural food colorants over a century in the East Asia (*3*, *4*). However, *Monascus* spp., except a few commercial strains, wild types, and mutants, have also been shown to coproduce a mycotoxin, citrinin (**Figure 1**), on some media under certain conditions (*5*, *6*). This is one of the prime reasons for the ban on the use of *Monascus* pigments in foods in the European Union and the United States. Recent studies have brought out the potential of pigment-producing genera, other than *Monascus*, that have been chemotaxonomically selected over those that are either known human pathogens or produce known mycotoxins (*1*, *7*−*9*). The color hues of such pigment-producing fungi have been shown to resemble and/or complement the color palette of contemporary natural food colorants in the red and yellow spectra (*7*). Polyketide pigments, such as *Monascus* pigments, are known to be unstable toward ultraviolet (UV) and visible light compared to their amino acid derivatives (*10*). Also, not much is known about the chemistry behind the color change of fungal polyketide pigments, including *Monascus* pigments, in food systems that are exposed to light. Therefore, this present study was undertaken with the purpose of studying the light stability of two

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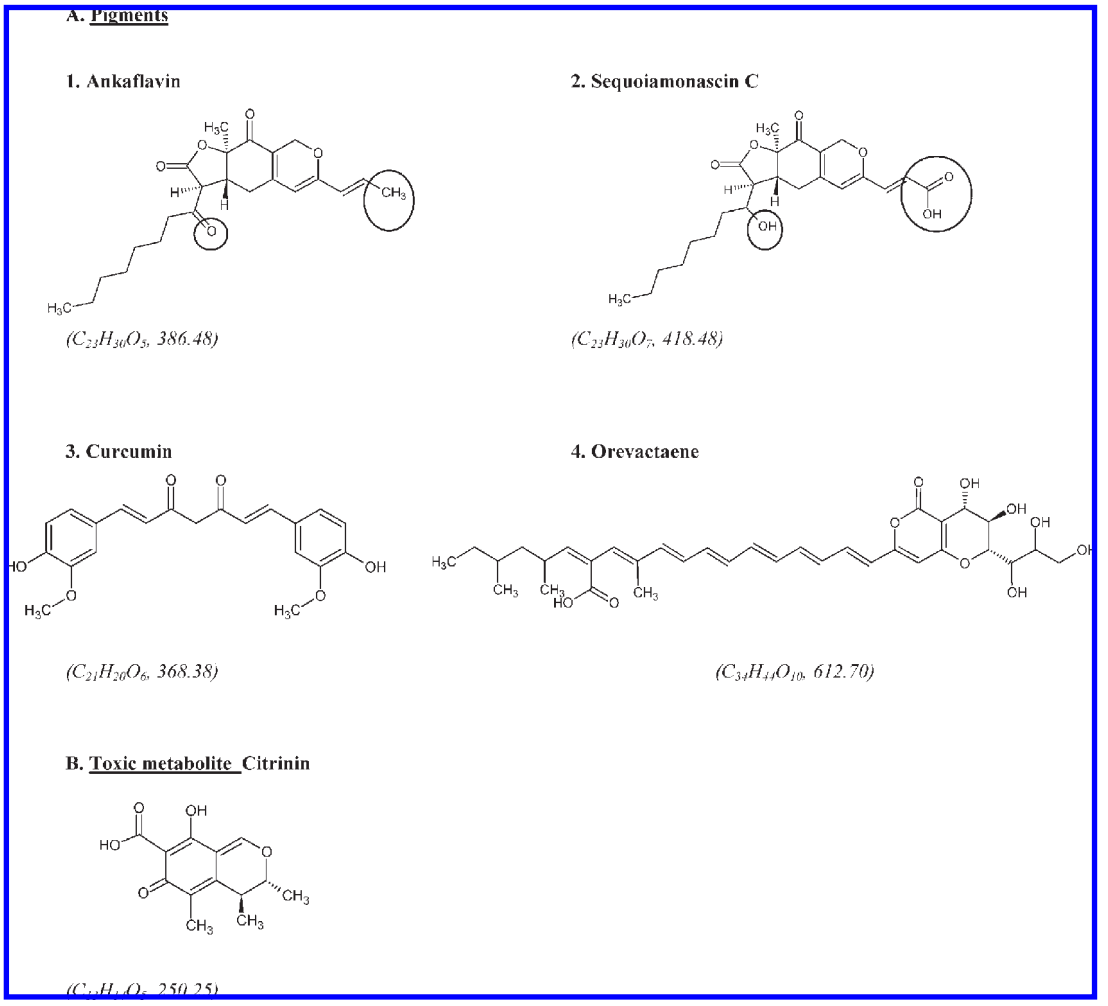

Figure 1. Structures of pigments relevant for the present work (the formula and nominal masses are in parentheses; highlighted areas in 1 and 2 show their structural difference; and 2 was detected and identified in the present work).

promising fungal pigment extracts characterized in our previous study (*7*). Natural colorants including fungal polyketide pigments are (in most cases) a mixture of several components, and therefore, it would be interesting to see the extent of stability of individual components toward light. We hypothesized that some of the components could be more photostable than the others and that their discriminative destabilization would affect the resultant color before and after the light exposure. In light of this, the quantitative and qualitative color change pattern of an orange−red and a yellow pigment extract produced by ascomycetous fungi belonging to the genera *Penicillium* and *Epicoccum*, respectively, was studied. We used an application-based approach, which is based on the CIELAB color space rather than the spectra, to assess the observed color change. A beverage-based medium, a soft drink medium typically used by the food colorant industry, was chosen as one of the food systems, in which the effect of pH could also be studied. Citrate buffer was used as another food system parallel to the soft drink medium because citrate is a commonly used preservative in many food products. The pigment composition in each of the two food model systems before and after the light exposure was analyzed by high-resolution liquid chromatography−diode array detection−mass spectrometry (LC−DAD−MS). The prime objective was to compare the photostability of the fungal pigment extracts to turmeric and a commercially available *Monascus* Red colorant used as controls for the yellow and orange−red colorants in our study.

MATERIALS AND METHODS

Preselection of Fungi, Media, Cultivation Conditions, and Food System Composition. Fungal isolates used in this study were procured from the IBT Culture Collection at the Center for Microbial Biotechnology, Technical University of Denmark, Kgs. Lyngby, Denmark. The fungal isolates were listed by the IBT numbers. *Penicillium aculeatum* IBT 14263 was cultivated on yeast extract sucrose (YES) agar, and *Epicoccum nigrum* IBT 41028 was cultivated on potato dextrose (PD) agar (*11*). The cultures were incubated in the dark at 25 °C for 7 days. Concentrated soft drink medium was kindly provided by Chr Hansen A/S (Hørsholm, Denmark). The concentrated medium contained (g/L): sucrose for soft drink (EU category 1, refined sugar), 430; sodium benzoate, food grade, 0.7; potassium sorbate, food grade, 0.9; citric acid, food grade, 8.6; and demineralized water, 559.8. This concentrated medium was then diluted 1:5 by demineralized water to obtain the working soft drink medium (SD). The resulting pH was 3.0 ± 0.2 with a °Brix level of 11.0 ± 1.0. The pH was changed using 0.1 M NaOH as per the need of the experiment. The model system of 0.1 M citrate buffer (CB) was prepared by mixing appropriate quantities of citric acid solution and sodium citrate solution until a desired pH was obtained.

Control Colorants. Water-soluble turmeric (product code T-PT8-WS) was kindly provided by Chr Hansen A/S (Hørsholm, Denmark), and *Monascus* Red colorant, designated as *Monascus* Red 3 (MR3), was a kind gift from Riken Vitamin Co. Ltd. (Japan).

Extraction of Fungal Pigments. Extraction was carried out by a modified version of the microextraction method (*12*): Larger plugs of 2.1 cm in diameter were taken out from fungal mycelia and the agar surface of the agar culture where the pigment was diffused. The plugs were quickly

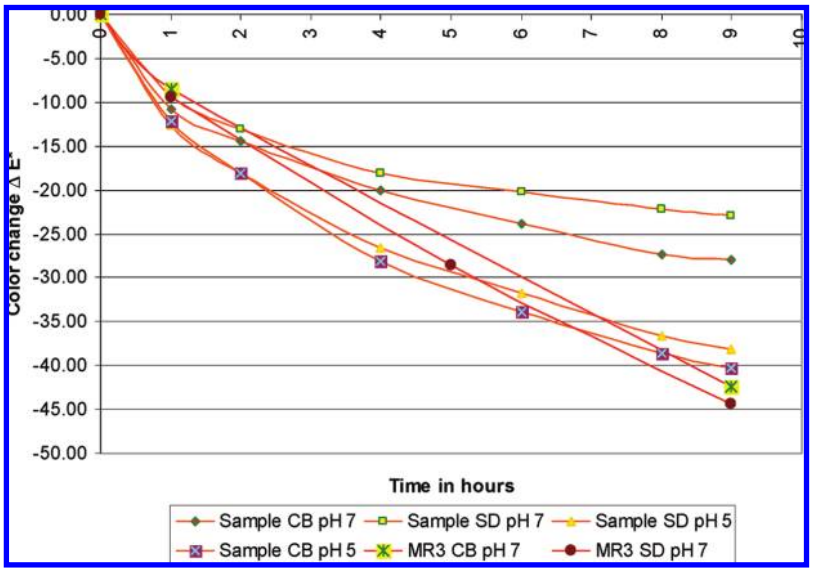

Figure 2. Quantitative decoloration over time of *P. aculeatum* IBT 14263 pigment extract (orange−red) in soft drink medium and 0.1 M citrate buffer at pH 5 and 7 with commercially available MR3 colorant as a control under light exposure (xenon lamp, light intensity/h 172.8 J/cm^2, cut-on wavelength 250 nm). The soft drink medium is designated as SD, and citrate buffer is designated as CB. The data for control for both CB and SD at pH 5 could not be recorded because it showed signs of degradation at 0 h itself. $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, where ΔL^* is a change in the lightness value with respect to time, Δa^* is a change in the red−green CIELAB coordinate a^* with respect to time, and Δb^* is a change in the yellow−blue CIELAB coordinate b^* with respect to time.

frozen using liquid nitrogen and transferred to a freezer (−18 °C) prior to their subsequent freeze-drying at −60 °C (Heto Drywinner, Holm and Halby, Denmark). The dried mass thus obtained was crushed in a pestle and mortar, and a coarse powder was obtained, which was stored in a freezer in closely sealed polythene bags. This powder was used for the extraction of pigments. A total of 1 g of fungal powder (from each species) was transferred to a vial, and 20 mL of 96% of ethanol was added. The vials were kept in an ultrasonic bath (Branson 2510, Kell-Strom, Wethersfield, CT) for 45 min. The extracted pigment was separated by filtration using Whatman filter paper number 1. The fungal residue was transferred back into the vial, and the extraction step was repeated until no more pigment could be extracted with lesser volumes of solvent (10 mL and subsequently with 5 mL). The crude pigment extract was collected in a round-bottom flask and dried in a rotary evaporator (Büchi Rotavapor R-134, Flawil, Switzerland) at 32 °C and with a pressure at the beginning of 300 mbar and gradually reducing it to 100 mbar. Because there should be no air in the flask, the vacuum in the flask with dried pigment residue was replaced with a stream of nitrogen to avoid air oxidation. The flask was closed tightly with a stopper, wrapped with a silver foil, and preserved in the freezer for subsequent use. For experiments, the dried pigment residue was redissolved in aqueous ethanol until it was completely soluble. This was then used as a stock for adding colorants to the food system.

Photostability Study. The colorants were added to 80 g of the two food systems until a desired lightness level (L value) was obtained. The L value was predetermined and kept in a range (see values range below) typically used by Chr. Hansen A/S for the soft drink medium based on their application experience. The L value was measured using a transmittance-based chromameter (Minolta CT 310, Konica Minolta, Mahwah, NJ). The initial lightness values used for the colorants were as follows: control MR3, 76.43 ± 0.50; pigment extract from *P. aculeatum* IBT 14263 on YES, 75.36 ± 0.24; turmeric, 97.67 ± 0.21; and pigment extract from *E. nigrum* IBT 41028 on PD, 97.8 ± 0.29. The sample solutions were transferred to canted neck flasks (IWAKI Scitech Division, Chiba, Japan, Asahi techno glass, 25 cm^2/canted neck) filled to the brim to prevent oxidation. The samples were put in a light cabinet (Suntest XLS+, Atlas-mts, Chicago, IL, xenon lamp, light intensity/h 172.8 J/cm^2, cut-on wavelength 250 nm) at 25 °C, with the position of the flasks noted (only 12 flasks could be fitted at a time). The light cabinet was programmed to run for a definite period of time after which the colorimetric values, L^*, a^*, and b^*, of aliquots from samples were noted using the chromameter. The process was continued until maximal decoloration. Also, the locations of the flasks in the light cabinet were interchanged to ensure a uniform exposure of light per sample.

Solid-Phase Extraction. Aliquots were withdrawn from the samples before and after the light exposure, and solid-phase extraction was performed as per our standardized method (*8*), with a slight modification in the washing step. Strata-X-C 33 μm cation mixed mode polymer columns (Phenomenex ApS, Allerød, Denmark) with a concentration of 60 mg/3 mL were used. A total of 1 mL of methanol was used for conditioning followed by 1 mL of distilled water for calibration. Samples, acidified with 0.1% phosphoric acid, were loaded and washed with 0.1% phosphoric acid and deionized water for citrate buffer and soft drink medium, respectively. Neutral and acidic components were eluted with methanol, and alkaline components were eluted with 5% (v/v) ammonia in methanol. The alkaline elute was added to acetonitrile with 50 ppm trifluoroacetic acid in a 1:5 ratio prior to chromatographic analysis considering the pH instability of the Luna C 18 II column (Phenomenex ApS, Allerød, Denmark) at alkaline pH.

Chromatographic Analysis. High-resolution LC−DAD−MS was performed on an Agilent HP 1100 liquid chromatograph (LC) system with a photodiode array detector (DAD) and a 50 × 2 mm inner diameter, 3 μm, Luna C 18 II column (Phenomenex ApS, Allerød, Denmark). The LC system was coupled to a LCT orthogonal time-of-flight mass spectrometer (Waters-Micromass, Manchester, U.K.) with a Z-spray electrospray ionization (ESI) source and a LockSpray probe and controlled by the MassLynx 4.0 software. The MS system was operated in the positive ESI mode using a water−acetonitrile gradient system, as described by Nielsen et al. (*13*).

Analysis of LC−DAD−MS Data. The colored components in the pigment extract were detected in the UV−vis chromatogram of 390−700 nm. The identification of sequoiamonascin C was based on both UV−vis and mass spectra from a total ion chromatogram (m/z 100−900) from positive ion electrospray. The UV−vis spectrum was obtained after background subtraction. The DAD−MS data for sequoiamonascin C and its proposed analogue is shown below:

Sequoiamonascin C was detected as m/z 419.20 $[M + H]^+$ and confirmed by the adduct m/z 482.22 $[M + Na + CH_3CN]^+$. The UV−vis spectrum was λ_{max}: 232 and 390. The proposed structural analogue was detected as m/z 419.21 $[M + H]^+$ and confirmed by the adduct m/z 436.19 $[M + NH_4]^+$ and 482.21 $[M + Na + CH_3CN]^+$. The UV−vis spectrum was λ_{max}: 244 and 398.

Table 1. Colorimetric Values for the Decoloration of the Orange−Red Fungal Extracts from *P. aculeatum* IBT 14263 and Control MR3 Colorant[a] in the Soft Drink Medium (SD) and 0.1 M Citrate Buffer (CB) as a Function of Time under the Light Exposure[b]

		time (h)							
color coordinates	sample name	0	1	2	4	5	6	8	9
*L**	14263 CB pH 5	75.24	77.72	80.62	85.90	nd[c]	88.32	90.91	91.62
	14263 CB pH 7	75.44	76.12	78.07	81.97	nd[c]	83.13	84.96	85.14
	14263 SD pH 5	75.64	78.75	81.5	86.15	nd[c]	88.17	91.01	91.86
	14263 SD pH 7	75.10	75.53	77.15	80.44	nd[c]	80.72	82.05	82.37
	MR3 SD pH 7	76.07	80.59	nd[c]	nd[c]	91.51	nd[c]	nd[c]	96.18
	MR3 CB pH 7	76.78	81.34	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]	96.21
*a**	14263 CB pH 5	31.01	24.13	19.82	13.44	nd[c]	10.63	7.38	6.36
	14263 CB pH 7	31.18	27.19	23.81	17.42	nd[c]	14.72	12.02	11.67
	14263 SD pH 5	30.37	23.45	19.63	14.15	nd[c]	11.82	8.5	7.54
	14263 SD pH 7	31.73	28.57	25.49	19.25	nd[c]	18.33	16.85	16.47
	MR3 SD pH 7	38.74	30.77	nd[c]	nd[c]	16.32	nd[c]	nd[c]	3.41
	MR3 CB pH 7	36.34	29.38	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]	3.38
*b**	14263 CB pH 5	39.38	29.75	26.27	20.31	nd[c]	15.67	13.25	12.16
	14263 CB pH 7	42.59	32.59	30.45	29.61	nd[c]	27.03	25.54	24.98
	14263 SD pH 5	37.55	27.39	24.14	19.17	nd[c]	15.12	12.46	11.54
	14263 SD pH 7	41.66	32.96	30.40	29.79	nd[c]	27.53	26.70	26.23
	MR3 SD pH 7	26.01	23.92	nd[c]	nd[c]	17.23	nd[c]	nd[c]	8.14
	MR3 CB pH 7	26.55	24.74	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]	8.18
*C**	14263 CB pH 5	48.29	38.31	32.91	24.35	nd[c]	18.94	15.17	13.72
	14263 CB pH 7	52.78	42.44	38.65	34.35	nd[c]	30.78	28.23	27.57
	14263 SD pH 5	48.29	36.06	31.11	23.83	nd[c]	19.19	15.08	13.78
	14263 SD pH 7	52.37	43.62	39.67	35.47	nd[c]	33.07	31.57	30.97
	MR3 SD pH 7	46.66	38.97	nd[c]	nd[c]	23.73	nd[c]	nd[c]	8.83
	MR3 CB pH 7	45.01	38.41	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]	8.85
*H**	14263 CB pH 5	51.03	50.95	52.97	56.51	nd[c]	55.85	60.88	62.39
	14263 CB pH 7	53.79	50.16	51.98	59.53	nd[c]	61.43	64.8	64.96
	14263 SD pH 5	51.03	49.43	50.88	53.57	nd[c]	51.98	55.70	56.84
	14263 SD pH 7	52.71	49.08	50.02	57.13	nd[c]	56.34	57.74	57.88
	MR3 SD pH 7	33.88	37.86	nd[c]	nd[c]	46.55	nd[c]	nd[c]	67.27
	MR3 CB pH 7	36.15	40.1	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]	67.55

[a] The data for control MR3 colorant for both CB and SD at pH 5 could not be recorded because it showed signs of degradation at 0 h itself. [b] Xenon lamp, light intensity/h 172.8 J/cm^2, cut-on wavelength 250 nm. [c] nd = not determined.

RESULTS AND DISCUSSION

The transmittances of each of the fungal extracts and the reference colorants were adjusted to a particular L^* value to provide for a fair comparison of colorants with respect to photostability. The 0 h L value range was decided on the basis of the optimal range of existing natural colorants in the soft drink medium that gave a desirable hue. For instance, the color of the orange−red pigment extract was adjusted to a L^* value of 75−77, and for the yellow pigment extract, it was in the range of 97−98. The color change was quantified using ΔE^* values, which is the difference in the color from a reference point in the three-dimensional CIELAB color space, where lightness is also considered along with colorimetric coordinates a^* (red−green parameter) and b^* (yellow−blue parameter) as seen from its formula given as $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, where ΔL^* is the change in the lightness value with respect to time, Δa^* is the change in the red−green CIELAB coordinate a^* with respect to time, and Δb^* is the change in the yellow−blue CIELAB coordinate b^* with respect to time. The reference point for each individual sample was the color recorded at time 0 of the light exposure.

Quantitative and Qualitative Color Change of Orange−Red Fungal Pigment Extract with MR3 as a Control under Light in Soft Drink Medium and Citrate Buffer at Two Different pH Values. The color change of the fungal pigment extract was relatively more rapid at pH 5 than at pH 7 in both of the liquid food model systems, with the soft drink medium (SD) being slightly better than the citrate buffer (CB) (**Figure 2**). The data for control for both CB and SD at pH 5 could not be recorded because it showed signs of degradation at 0 h itself. The relatively slower decoloration of fungal pigment extract observed in the soft drink medium might be due to stabilizing effects by some unknown mechanism conferred by the other ingredients in the soft drink medium, e.g., sucrose, sodium benzoate, or potassium sorbate. Also, the magnitude of decoloration was more in the first 2 h for the two systems especially noticeable at pH 5 (**Figure 2**), indicating a classical first-order decay mechanism underlying the color change. It has been recently shown that the pigment extract obtained from *P. aculeatum* IBT 14263 on YES medium had *Monascus* pigments and their derivatives, such as monascorubrin (orange), xanthomonasin A (yellow), and a threonine derivative of rubropunctatin (purple−red) (*9*). This might explain the relatively higher light stability of these pigment extracts at pH 7 because *Monascus* pigments in solution are more stable at near neutral and/or alkaline pH (*14*, *15*). In comparison to the control *Monascus* red colorant MR3 at pH 7, the color change of the orange−red pigment extract in both the food systems at pH 7 was less pronounced, especially after 4 h (**Figure 2**). This could be due to the fact that orange−red pigment extracts had relatively more yellow components than the control *Monascus* red colorant MR3. Because it is previously reported that the red pigments of *Monascus* are less stable than the yellow pigments in solution (*14*), the continual decoloration of the control *Monascus* red colorant MR3 at pH 7 in the two tested food systems can be explained. Thus, it can be inferred that the relatively larger proportion of the

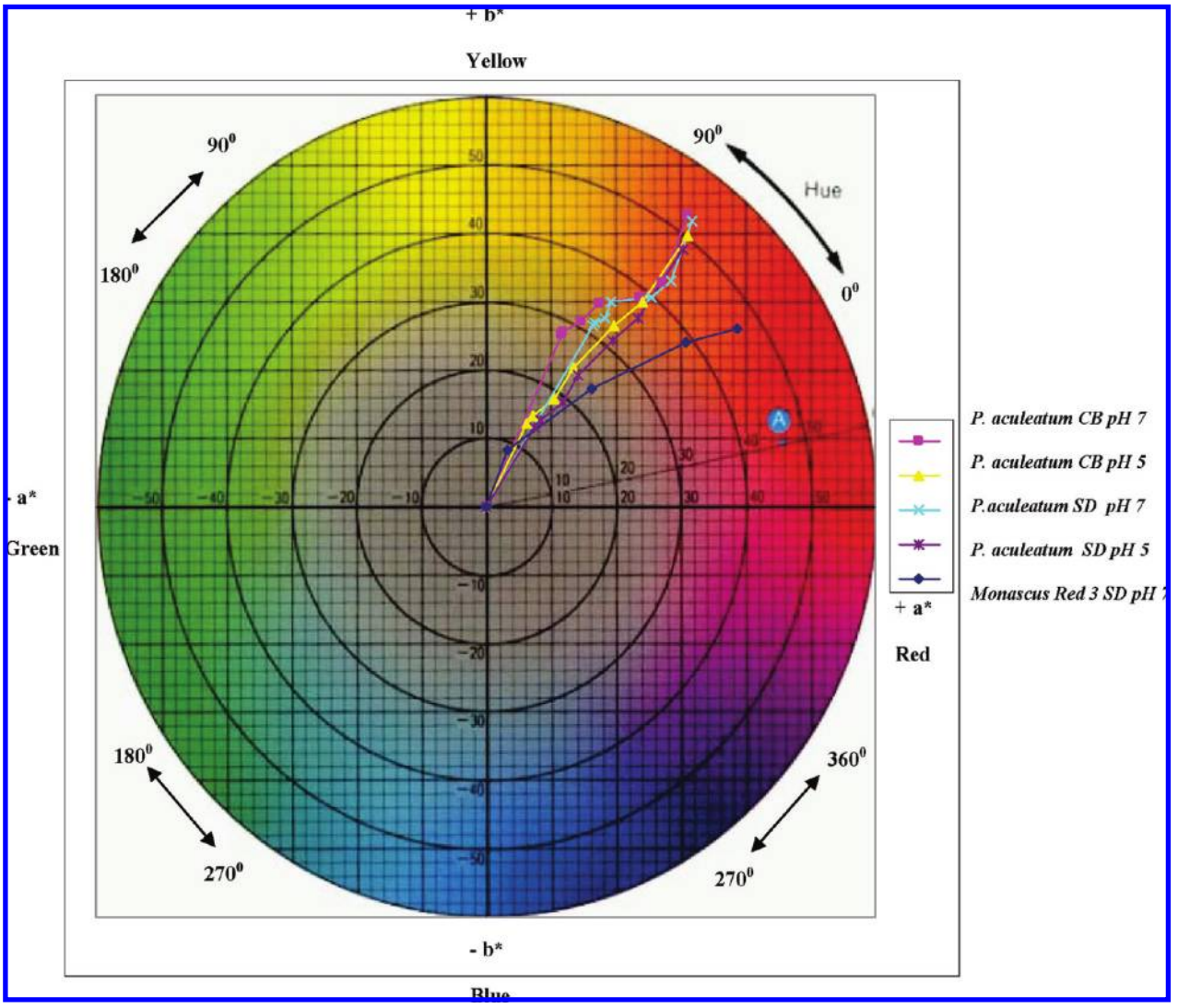

Figure 3. Qualitative color change over time in CIELAB color space for *P. aculeatum* IBT 14263 pigment extract (orange−red) in soft drink medium and 0.1 M citrate buffer at pH 5 and 7 with commercially available MR3 colorant as a control under light exposure (xenon lamp, light intensity/h 172.8 J/cm^2, cut-on wavelength 250 nm). The soft drink medium is designated as SD, and citrate buffer is designated as CB. The data for control for both CB and SD at pH 5 could not be recorded because it showed signs of degradation at 0 h itself.

yellow components in the orange−red pigment extract was the factor that determined its photostability in the two tested food systems at pH 7. In the future, it should be possible to obtain tailor-made but natural yellow fungal colorants by manipulating media and culture conditions.

Table 1 and **Figure 3** illustrate a more qualitative aspect of decolorization: how the color hues of the orange−red pigment extracts and the control MR3 differed initially and after the light exposure as a function of time. The *a** and *b** values of the control were relatively higher and lower, respectively, compared to all of the sample pigment extracts, resulting in a hue angle of 34, indicating more red hue than orange as in the case of the sample pigment extracts (**Table 1** and **Figure 3**). The hue values of the pigment extract in both the food systems at pH 5 and 7 did not change much during the first 2 h, and then the path toward a weaker color was changed (**Figure 3**). The pigment extract in both the food systems at pH 5 did not show as much deviation as the pigment extract in the two food systems at pH 7 (**Figure 3**). Hence, at pH 7, a slight increase in the hue angles of the color in both of the two food systems at pH 7 was seen (**Table 1** and **Figure 3**), indicating a more yellow color hue. This could be due to the degradation of the red components, as explained earlier. This result is in agreement with the result obtained by Jung et al. (*10*) in the case of the decoloration of control red *Monascus* pigments under sunlight irradiation.

Chemistry of Photodegradation of the Orange−Red Pigment Extract. To see and understand the photodegradation pattern of the most photostable system, i.e., the soft drink medium at pH 7, the pigment composition of the two food systems before and after the light exposure was analyzed by LC−DAD−MS. There were two minor orange−red-colored components and two yellow components present with characteristic UV−vis spectra in the soft drink medium at pH 7 before the light exposure (**Figure 4A**). Among the yellow components, there was a major peak eluting at the retention time (RT) of 10.45 min and a minor peak with RT of 11.00 min (**Figure 4A**). After the light exposure, the peaks indicating the orange−red pigments disappeared, indicating that these minor orange−red components degraded to other compounds. In the case of yellow components, the height of the major peak with RT of 10.44 min also decreased after light exposure, while the height of the minor peak with RT of 10.99 min increased (**Figure 4B**). The yellow major peak with RT of 10.45 min was identified to be sequoiamonascin C (panels **A** and **C** of **Figure 4**) on the basis of the mass and the UV−vis spectra (panels **A1**/**B1**

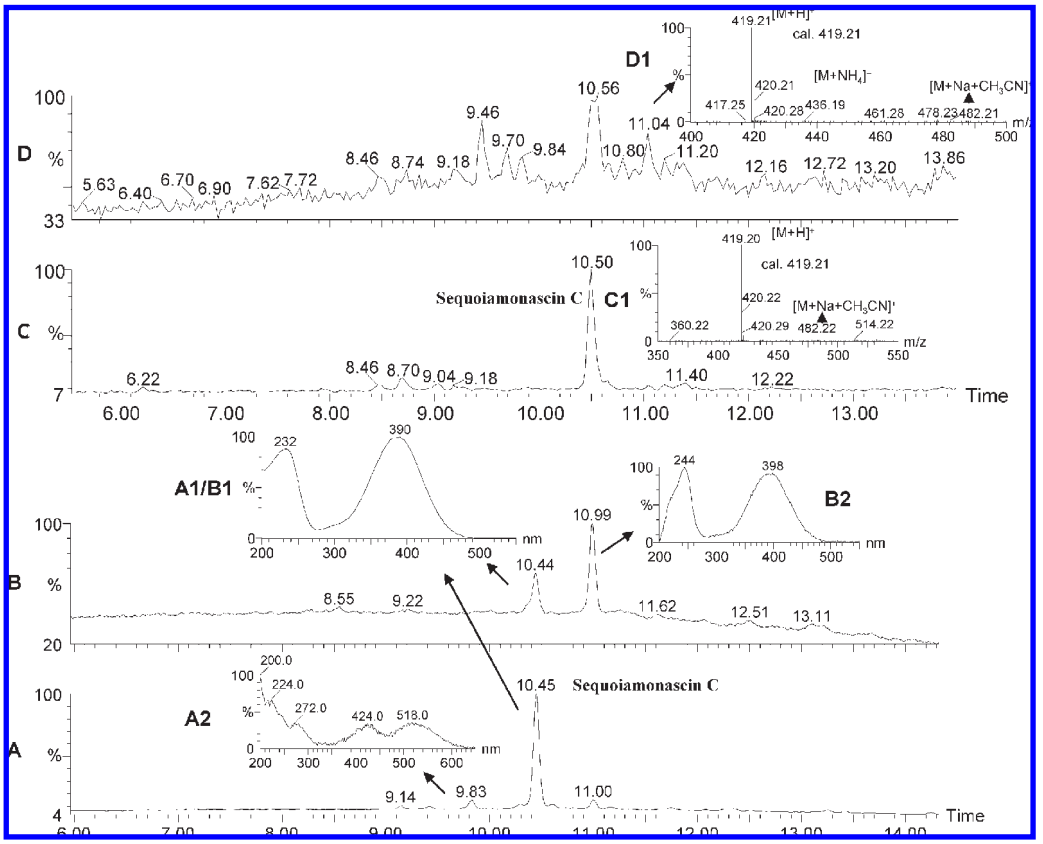

Figure 4. Bottom panel (A and B) depicts UV−vis chromatograms of 390−700 nm of a neutral/acidic fraction of the pigment extract obtained from *P. aculeatum* IBT 14263 on YES medium in soft drink medium at pH 7. (A) UV−vis chromatogram before the light exposure. (B) UV−vis chromatogram after the light exposure. A1/B1 shows the UV−vis spectrum of sequoiamonascin C. A2 shows the UV−vis spectrum of one of the orange−red components. B2 represents the UV−vis spectrum of the proposed structural analogue of sequoiamonascin C. Top panel (C and D) depicts total ion chromatograms (*m*/*z* 100−900) from positive ion electrospray of the same fraction. (C) Total ion chromatogram (*m*/*z* 100−900) before the light exposure. (D) Total ion chromatogram (*m*/*z* 100−900) after the light exposure. C1 depicts the mass spectrum of sequoiamonascin C. D1 represents the mass spectrum of its structural analogue.

and **C1** of **Figure 4**). Sequoiamonascin C belongs to a series of fungal metabolites, sequoiamonascins A−D, which were previously reported to be produced by *Aspergillus parasiticus* (*16*). The UV−vis and mass spectral data of sequoiamonascin C matched quite well with the reported data (*16*) and the data available in the database, such as Antibase (Wiley-VCH, Weinheim, Germany). The structure of sequoiamonascin C (**Figure 1**) revealed its similarity to a related polyketide-based *Monascus* pigment ankaflavin with the difference being in the −COOH moiety instead of a methyl group attached to the six-membered ring and the presence of a hydroxyl group instead of a keto group in the aliphatic chain, as highlighted in **Figure 1**. This structural similarity may explain the similarity of their UV−vis spectra. The minor peak, RT of 11.00 min, present in **Figure 4A** is envisaged to be a structural homologue of sequoiamonascin C because it had the same mass spectrum but slightly different UV−vis spectrum (**Figure 4B2**). Sequoiamonascin C, without its structural analogue, was also seen in the crude pigment extract alone (data not shown). Sequoiamonascin D is reported to be produced by an unidentified species of *Penicillium* (*17*). This is a first ever report of sequoiamonascin C to be produced by *P. aculeatum* IBT 14263 on YES medium and brings out the discovery of yet another *Monascus*-like pigment in *P. aculeatum* IBT 14263. The chemistry behind the presence of the structural homologue of sequoiamonascin C in the soft drink medium at pH 7 and their interconversion upon light exposure can be explained as follows: Sequoiamonascin C has a log *D* value (used as the lipophilicity index) of −0.24 at pH 7 (SciFinder Scholar Compound registry number 561305-71-3), meaning that it is quite hydrophilic but at the same time its low pK_a value (4.84) resulted in its ionization in the soft drink medium at pH 7, giving rise to its structural analogue that co-existed before the light exposure. The light exposure induced a modification in the structure possibly with regard to the arrangement of the conjugated double bonds, as exhibited by the resulting slight change in the UV−vis spectrum (**Figure 4B2**). The weaker color after the light exposure was due to the presence of only one major yellow-colored component, with the area underlying the visible spectrum being small. Also, a few tiny peaks were observed after the light exposure (**Figure 4B**). These peaks could be the degradation products of the red−orange components present before the light exposure. Similar results were obtained in the case of citrate buffer at pH 7, confirming the pH effect.

Quantitative and Qualitative Color Change of Yellow Fungal Pigment Extract with Turmeric as a Control under Light in the Soft Drink Medium and Citrate Buffer at Two Different pH Values. Figure 5 shows that the magnitude of color change of the pigment extract obtained from *E. nigrum* IBT 41028 on PD in the two food systems was not as large as compared to the orange−red pigment extract with respect to the change in pH of the food systems. Also the two food systems did not differ much from each other with respect to the extent of photo-decolorization. The striking feature was that the decoloration was not as pronounced as in the control extract of turmeric, which decolorized (**Figure 5**) significantly after 1 h. Turmeric, the color of which mainly comes from the compound curcumin (**Figure 1**), is one of the weakest light-stable

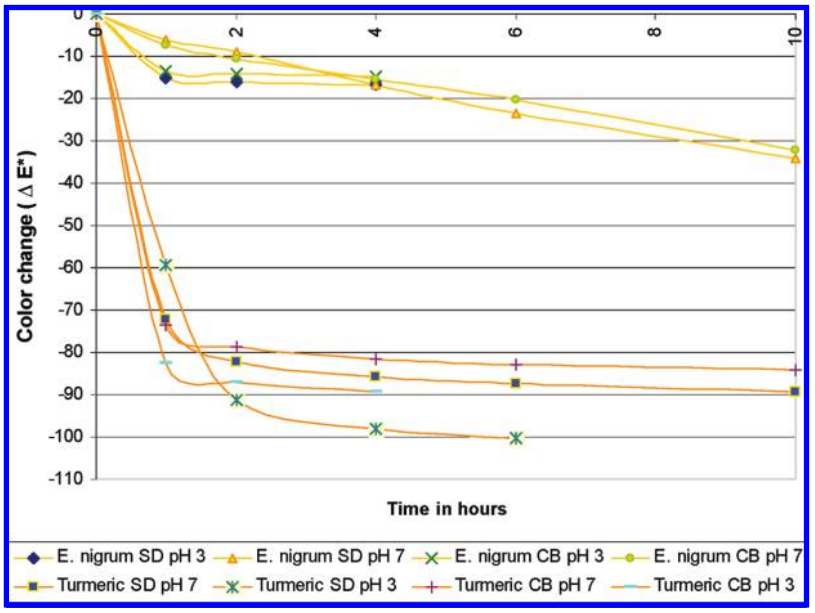

Figure 5. Quantitative decoloration over time of *E. nigrum* IBT 41028 pigment extract (yellow) in soft drink medium and 0.1 M citrate buffer at pH 5 and 7 with commercially available turmeric colorant as a control under light exposure (xenon lamp, light intensity/h 172.8 J/cm^2, cut-on wavelength 250 nm). The soft drink medium is designated as SD, and citrate buffer is designated as CB. $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, where ΔL^* is the change in the lightness value with respect to time, Δa^* is the change in the red−green CIELAB coordinate a^* with respect to time, and Δb^* is the change in the yellow−blue CIELAB coordinate b^* with respect to time.

Table 2. Colorimetric Values for the Decoloration of the Yellow Fungal Extract from *E. nigrum* IBT 41028 and Control (Turmeric) in 0.1 M Citrate Buffer (CB) as a Function of Time under the Light Exposure[a]

		time (h)					
color coordinates	sample name	0	1	2	4	6	10
L^*	turmeric CB pH 3	97.94	99.55	99.61	99.68	[b]	[b]
	E. nigrum CB pH 3	98.17	99.42	99.46	99.54	[b]	[b]
	turmeric CB pH 7	97.73	98.63	98.67	98.59	98.60	98.40
	E. nigrum CB pH 7	97.45	98.09	97.80	98.57	98.77	99.23
a^*	turmeric CB pH 3	−20.72	−3.73	−1.94	−1.16	[b]	[b]
	E. nigrum CB pH 3	−3.26	−0.58	−0.45	−0.35	[b]	[b]
	turmeric CB pH 7	−19.86	−4.11	−2.33	−1.49	−1.01	−0.48
	E. nigrum CB pH 7	−11.33	−10.67	−10.00	−9.01	−7.75	−4.03
b^*	turmeric CB pH 3	91.17	10.28	6.01	3.98	[b]	[b]
	E. nigrum CB pH 3	16.54	3.24	2.64	2.07	[b]	[b]
	turmeric CB pH 7	86.87	15.00	10.02	7.40	6.12	4.84
	E. nigrum CB pH 7	43.79	36.56	33.30	28.56	23.85	12.30
C^*	turmeric CB pH 3	93.49	10.94	6.32	4.15	[b]	[b]
	E. nigrum CB pH 3	16.86	3.29	2.68	2.10	[b]	[b]
	turmeric CB pH 7	89.11	15.55	10.29	7.55	6.20	4.86
	E. nigrum CB pH 7	45.23	38.09	34.77	29.95	25.08	12.94
H^*	turmeric CB pH 3	102.8	109.94	107.89	106.25	[b]	[b]
	E. nigrum CB pH 3	101.15	100.15	99.67	99.60	[b]	[b]
	turmeric CB pH 7	102.88	105.32	103.09	101.38	99.37	95.66
	E. nigrum CB pH 7	104.51	106.27	106.72	107.51	108.00	108.14

[a] Xenon lamp, light intensity/h 172.8 J/cm^2, cut-on wavelength 250 nm. [b] The color was too faint to be measured.

colorants currently being used and thus needs to be encapsulated (*18*).

Table 2 and **Figure 6** depict the qualitative change in the color over the time in citrate buffer at two different pH values. It is important to note that the initial a^*, b^*, and C^* values of the control (turmeric) for both pH 3 and 7 were significantly higher than the *E. nigrum* extracts (**Table 2**), even though the L^* values were adjusted to the relatively similar level. We ascribe this difference to the possibly higher extinction coefficient and pH stability of curcumin, the major component of the turmeric extract, than orevactaene. However, the a^*, b^*, and C^* values of turmeric dropped drastically after 1 h of light exposure compared to the extracts of *E. nigrum* (**Table 2**), indicating a weaker photostability of turmeric extract. The color hues, on the other hand, did not change significantly (especially at 0 h) and were found to be similar for the extracts of *E. nigrum* as well as the control (**Table 2** and **Figure 6**). The color of the *E. nigrum* pigment extract at pH 3 was much weaker than at pH 7 at 0 h and thus faded after 4 h (**Table 2**). This could be due to the effect of the concentration because it was not possible to have an equimolar concentration of pigments in the two food systems considering the crude nature of the fungal pigment extract. Nevertheless, the data signify the potential of *Epicoccum* pigment extract as a photostable colorant.

It must be noted that the intensity of irradiation/h in the cabinet (172.8 J/cm^2) that we used for our experiments was 30−40 times higher than the regular intensity of sunlight irradiation/h in Denmark (personal communication, Paul Eriksen, Danish Meteorological Institute, Dec 2008). Also, the intensity of light used under storage conditions of food products could be even lower. This implies that the time for decoloration in the real scenario would be much longer than the current experimental findings. Jung et al. (*10*) reported the enhanced photostability of amino acid derivatives of *Monascus* pigments under both UV light (365 nm and 8 W) and outdoor sunlight irradiation, with the average intensity of sunlight irradiation/h ranging from 230 to 313 J/cm^2, which is higher than our experimental value. They found the amino acid derivatives of *Monascus* pigments much more stable compared to their parent red pigment under UV light than under sunlight possibly because of the lower irradiation strength of the UV light than sunlight that they used. Because wavelength distribution of sunlight irradiation was not mentioned by Jung et al. (*10*), it is thus difficult to directly correlate our results with the results obtained by them. Nevertheless, it is very likely that *Monascus* pigments and their amino acid

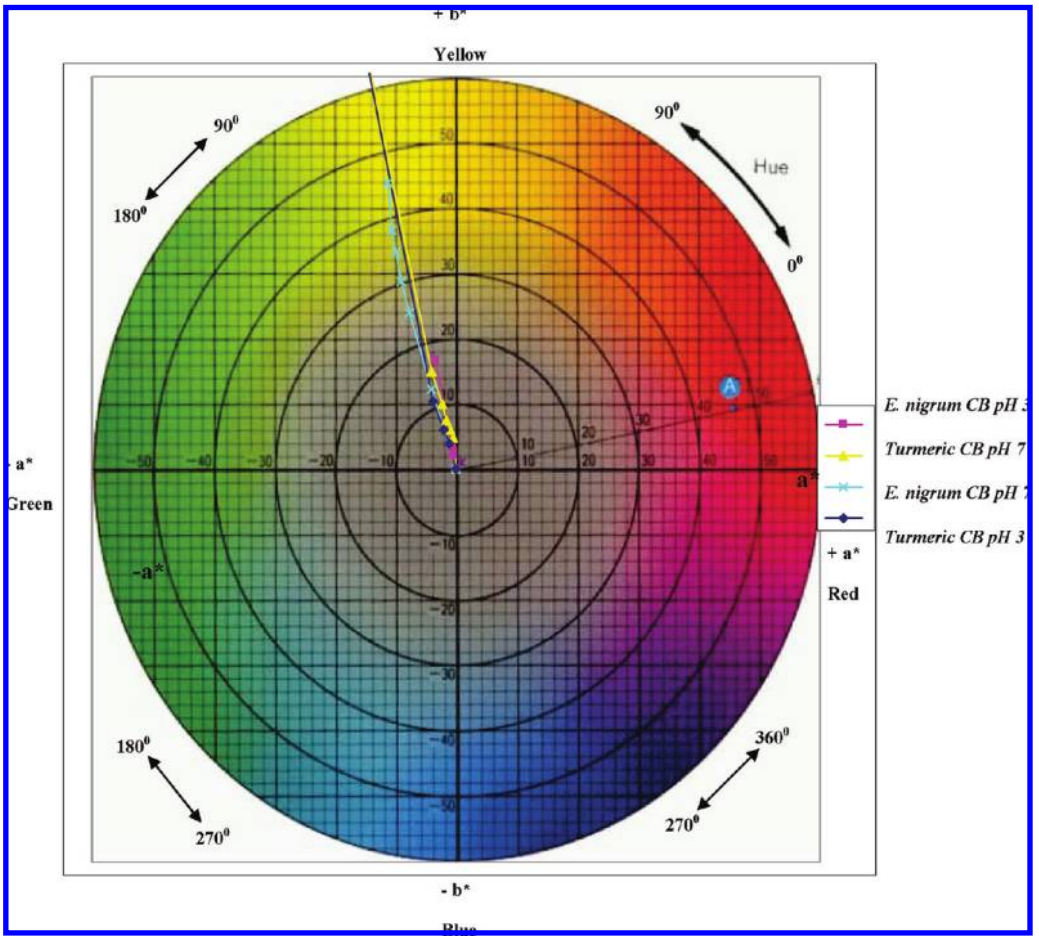

Figure 6. Qualitative color change in CIELAB color space of *E. nigrum* IBT 41028 pigment extract (yellow) in 0.1 M citrate buffer at pH 3 and 7 with commercially available turmeric colorant as a control under light exposure (xenon lamp, light intensity/h 172.8 J/cm^2, cut-on wavelength 250 nm). Citrate buffer is designated as CB.

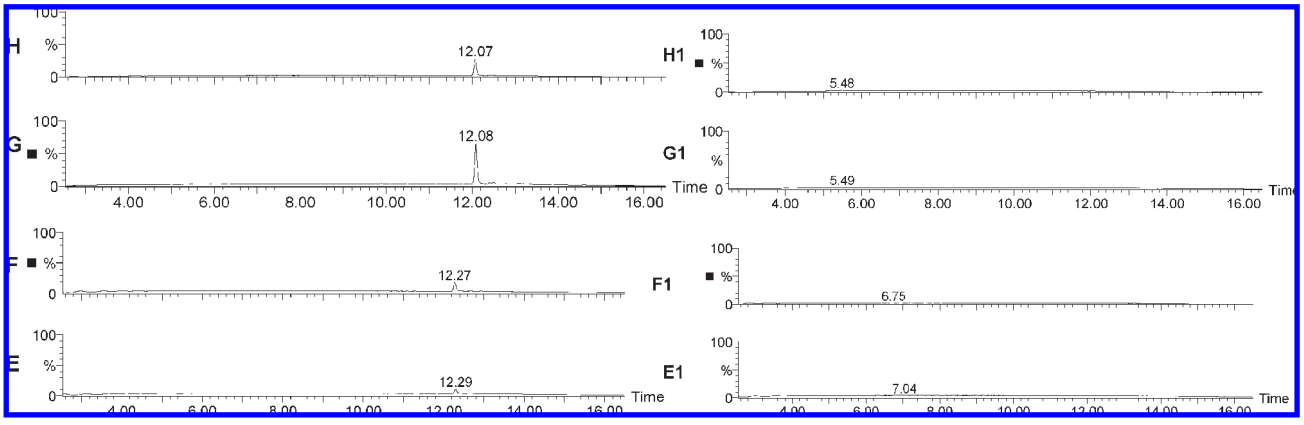

Figure 7. UV−vis chromatograms of 390−700 nm of pigment extract obtained from *E. nigrum* IBT 41028 on PD medium in 0.1 M citrate buffer at pH 3 and 7. Data normalized to the intensity of 1.0 × 10^6 to focus on the major peak orevactaene. (E) Presence of orevactaene in citrate buffer at pH 3 neutral/acidic elute before the light exposure. (E1) Absence of orevactaene after the light exposure. (F) Presence of orevactaene in citrate buffer at pH 3 alkaline elute before the light exposure. (F1) Absence of orevactaene after the light exposure. (G) Citrate buffer at pH 7 neutral/acidic elute before light exposure. (G1) Absence of orevactaene after the light exposure. (H) Presence of orevactaene in citrate buffer at pH 7 alkaline elute before the light exposure. (H1) Absence of orevactaene after the light exposure.

derivatives may be more photostable than some of the existing natural colorants. Thus, the relatively higher photostability of both of the fungal pigment extracts compared to the controls is worth considering. Notably, the color hue of turmeric is similar to the pigment extract of *E. nigrum* IBT 41028 on PD, as previously described (*7*). The yellow *Epicoccum* pigment extract thus harbors potential additional yellow colorants with better photostability.

Chemistry of Photodegradation of the Pigment Extract Obtained from *E. nigrum* IBT 41028 on PDA. The color of the pigment extract of *E. nigrum* IBT 41028 on PD was predominantly due to the presence of an oxopolyene polyketide orevactaene (**Figure 1**), as

reported previously (*19*). The effect of light on the degradation of *Epicoccum* pigment extract in citrate buffer at pH 3 and 7 was studied by chromatographically analyzing the pigment composition before and after the light exposure. Orevactaene is relatively more hydrophilic at pH 7 because its log *D* value at pH 7 is 1.17 (SciFinder Scholar Compound registry number 197631-20-2). Also, being highly acidic (pK_a 4.07 ± 0.19), it ionized more at pH 7 and was eluted more in the neutral/acidic than in the alkaline elute (indicated by its higher peak height) (panels **G** versus **H** of **Figure 7**). However, the compound is quite hydrophobic at pH 3 (log *D* value at pH 3 is 4.01, SciFinder Scholar Compound registry number 197631-20-2) and remained in un-ionized form as a result; a relatively small peak is detected in both neutral/acidic and the alkaline elute. This might explain the lower chroma of the *Epicoccum* pigment extracts at pH 3. The retention time also changed from 12.07/12.08 to 12.27/12.29, which could be due to the change in the relative abundance of orevactaene isotopes. The effect of pH was also observed in the soft drink medium. After the light exposure, the absence of orevactaene could be seen in both neutral/acidic elute and the alkaline elute extracted from citrate buffer at pH 3 and 7 (panels **E1**−**H1** of **Figure 7**).

In conclusion, we have shown two fungal pigment extracts exhibiting enhanced photostability compared to the controls in our study. The application-based approach was shown to add a more realistic dimension in assessing the photostability using CIELAB color space. Yellow components of the orange−red pigment extract from *P. aculeatum* IBT 14263 were found to be more light-stable than the red components. In addition, yet another *Monascus*-like pigment sequoiamonascin C was discovered in the extract of *P. aculeatum* IBT 14263 on YES medium. The data indicate that ascomycetous fungi may provide a significant source of new, natural food colorants with desirable functionalities that may even surpass those of currently used natural colorants.

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