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Effect of ohmic heating processing conditions on color stability of fungal pigments

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Abstract

The aim of this work was to analyze the effect of ohmic heating processing conditions on the color stability of a red pigment extract produced by *Penicillium purpurogenum* GH2 suspended in a buffer solution (pH 6) and in a beverage model system (pH 4). Color stability of pigmented extract was evaluated in the range of 60–90 °C. The degradation pattern of pigments was well described by the first-order (fractional conversion) and Bigelow model. Degradation rate constants ranged between 0.009 and 0.088 min^{-1} in systems evaluated. Significant differences in the rate constant values of the ohmic heating-treated samples in comparison with conventional thermal treatment suggested a possible effect of the oscillating electric field generated during ohmic heating. The thermodynamic analysis also indicated differences in the color degradation mechanism during ohmic heating specifically when the pigment was suspended in the beverage model system. In general, red pigments produced by *P. purpurogenum* GH2 presented good thermal stability under the range of the evaluated experimental conditions, showing potential future applications in pasteurized food matrices using ohmic heating treatment.

Keywords

Fungal pigments, ohmic heating, stability, mathematical modeling

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INTRODUCTION

Color plays an important role in the acceptability of foodstuffs and is a basic aspect of the identification, assessment, and judgment of quality. Also, it is the sensorial attribute mostly employed by the food industry to get the attention of consumers in order to increase products sales (Vendruscolo et al., 2013). Consequently, most of the food industries add colorants to final products in order to enhance/replace color lost and to minimize product variations during processing allowing to obtain homogeneous and high-quality final products (Aberoumand, 2005).

Recently, research about natural pigments has risen due to the growing demands for natural products and also due to the association of synthetic dyes with toxic effects on health (Mapari et al., 2005). Natural pigments can be extracted or produced by different sources such as plants; animals; minerals; and some microorganisms such as yeast, bacteria, microalgae, and fungi (Dufossé, 2006). However, microbial pigments have some advantages over plant-extracted pigments

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such as the absence of seasonal impediments and could be produced in high yields (De Carvalho, 2004).

Recently, filamentous fungi have been reported as potential sources of pigments with different chemical structures and color hues (Mapari et al., 2009). To date, the most well-known fungal pigments producer is *Monascus* strains. For hundreds of years, *Monascus* pigments have been used as food colorants in Asia, mainly in China and Japan. However, a disadvantage of pigmented extracts (EXs) produced by *Monascus* is the coproduction of the mycotoxin citrinin (Mapari et al., 2010).

Recently, it was reported the ability of *Penicillium* strains including *Penicillium purpurogenum* species to produce polyketides azaphilone pigments similar to *Monascus* without synthesis of citrinin or other mycotoxins making it a potential pigment producer strain for future applications in food (Dufossé et al., 2014; Mapari et al., 2008, 2009, 2010).

The implementation of these natural pigments at industrial scale requires detailed knowledge of certain aspects such as their stability under different processing conditions, which will allow an optimal process design and optimization (Fernández-López et al., 2013). In food processing areas, the stability is considered one of the critical aspects for application in a real food system (Rawson et al., 2011).

Currently, conventional thermal processing is mostly used in food industry to ensure the microbiological quality of food and enzyme inactivation (Sarkis et al., 2013). Ohmic heating (OH) technology is a promising alternative to conventional heating (CH) technology having the advantage to reach high temperatures in a short period, also allowing rapid and uniform heating (Bansal and Chen, 2006; De Alwis and Fryer, 1992; Stirling, 1987). By not requiring extensive processing times, it allows reducing the damage and degradation of thermolabile compounds resulting in an improvement in quality parameters' retention and sensorial properties (Bozkurt and Icier, 2012; Guida et al., 2013). OH technology has been applied during the pasteurization of fruit juices in order to inactivate some quality-related enzymes (Lee et al., 2015; Samaranayake and Sastry, 2016a, 2016b). Regarding the stability of food colorants under OH, some researchers have studied the effect of OH process conditions in anthocyanins degradation in acerola pulp (Mercali et al., 2013), blueberry pulp (Sarkis et al., 2013), jaboticaba juice (Mercali et al., 2015) grapefruit and blood orange (Achir et al., 2016). However, studies regarding the effect of OH technology on microbial pigments stability have not been carried out yet.

The aim of this work was to analyze the effect of OH processing conditions on the color stability of a red pigment EX produced by *P. purpurogenum* GH2 suspended in a buffer solution and in a beverage model system (MS) and to compare this effect with CH processes.

MATERIALS AND METHODS

Microorganism

P. purpurogenum GH2 strain provided by the Food Research Department (DIA-UAdeC) was used for pigment production. This fungal strain was isolated from Mexican desert plants of Coahuila, characterized, purified, and stored in the culture collection. The microorganism was maintained on potato dextrose agar (PDA) slants at 4 °C and subcultured periodically.

Culture media

The inoculum was grown in potato dextrose broth (ATCC medium: 336). This was prepared by boiling 300 g of little diced potato in 500 ml of water until properly cooked. Then, the supernatant and potatoes were filtered through cheesecloth and distillate water was added to complete 1.0 l. Finally, 20.0 g of glucose was added before sterilization. Czapek–Dox modified medium was used as fermentation culture medium for pigment production and prepared according to Méndez-Zavala (2011) and Morales-Oyervides et al. (2015) containing (g/l): D-xylose 15.0, $NaNO_3$ 3.0, $MgSO_4{\cdot}7H_2O$ 0.5, $FeSO_4{\cdot}7H_2O$ 0.1, K_2HPO_4 1.0, KCl 1.0, and ethanol 20.0.

Cultivation

First, *P. purpurogenum* GH2 strain was grown onto plates with 30 ml of PDA medium and incubated at 30 °C for five days (INO 650V-7, New Brunswick, USA). After, a spore's suspension was collected by adding tween 20 solution (0.01% v/v). Inoculum (mycelium) was prepared by inoculating Erlenmeyer flasks containing potato dextrose broth with 1×10^5 spores/ml and incubated at 30 °C and 200 r/min for three days in an orbital shaker (Inova 94, New Brunswick Scientific, USA). Pigment production was carried out according to conditions reported by Morales-Oyervides et al. (2015). Briefly, Erlenmeyer flasks containing Czapek–Dox modified medium were inoculated with 10% (v/v) of mycelium and incubated at 30 °C and 200 r/min during six days.

Extracellular pigment extraction

Extracellular pigment recovery was carried out according to the methodology previously reported by Méndez-Zavala (2011) and Morales-Oyervides et al. (2015). The aqueous EX was centrifuged at 10,000 r/min

and 4 °C (Sigma-18KS, Germany) during 20 min. Finally, the EX was filtrated through 0.45 μm cellulose membrane filter (Millipore, USA).

Pigment determination

The red pigment produced during fermentation was quantified by optical density at 500 nm using a spectrophotometer (Unico UV 2150, USA) according to the maximum absorbance wavelength previously determined by scanning the maximum sensitivity at the light absorption for the presence of the pigment. Different blank references to pigment quantification were needed. The Czapek–Dox modified medium was used as a blank reference to quantify the amount of pigment produced by fermentation. During pigments degradation as a result of processing, a citrate–phosphate buffer of pH 6.0 and 4.0 with the components of the MS was used as a blank reference for the red pigment EX and the beverage MS, respectively. For this work, the red color intensity is the critical quality factor of interest.

Samples preparation

Red pigments EX. The red pigment EX of *P. purpurogenum* GH2 was used to prepare pigment solutions adjusting the absorbance around 1.0 unit at 500 nm. The EX was diluted in citrate–phosphate buffers to maintain the pH value of 6.0 during processing with a conductivity of 17.30 mS cm^{-1} (Hanna, USA) (Silveira et al., 2013).

Model beverage system. The model beverage system was based on the formulation previously reported by Dyrby et al. (2001) with some modifications. Model beverage composition was as follows (g/l): sucrose 110.0, citric acid 5.0, potassium sorbate 0.18, and glutamine 0.057. The color was adjusted by adding the pigment EX in order to establish color intensity around 1.0 absorbance units at 500 nm. The MS was kept at pH 4.0 and conductivity (Hanna, USA) of 7.28 mS cm^{-1} during different treatments using a citrate–phosphate buffer.

OH process

The experiments were performed in a glass stirred reactor with an OH system using a frequency of 60 Hz. The OH unit consisted of a variable power amplifier (Superior Electric, Farmington, Connecticut, USA) attached to a data acquisition system (PicoLog TC-08, Pico Technology, UK) to monitor temperature (Thermocouple type K) and voltage (Multimeter, RS232C, Mul-600, Mexico).

The thermocouple was collocated in the center of the vessel to obtain a uniform measurement.

The ohmic cell consisted of a 260 ml Pyrex glass vessel provided with a water jacket. The vessel height was 13.0 cm and 7.0 cm wide. The electrodes were made of stainless steel with a diameter of 4.7 cm and 5.0 cm of height according to the vessel design. The distance between electrodes in the cell was 4 cm.

The kinetic experiments were conducted at different temperatures (60, 70, 80, and 90 °C). Samples were withdrawn at various heating times (0, 15, 30, 45, 60, and 75 min) and transferred to an ice water bath (Precision 183, USA) for subsequent optical density measurement. The samples were stirred during all treatment times by means of a magnetic stirrer plate (Thermo Scientific, USA) in order to avoid temperature gradients in the treatment chamber.

An extended processing time is needed to get important information regarding pigment stability even though in practice, only a few seconds or minutes of heating at high temperatures are necessary to get a successful pasteurization or sterilization. Therefore, these conditions (time and temperature) were chosen in order to obtain a significant decrement in pigment concentration during the process and fit the data to a kinetic model without overestimating model parameters.

For the OH process, the sample temperature was increased by applying 20 V to the pigmented EX suspended in a buffer solution and 30 V for the beverage MS until reaching the target temperature. When the sample reached the desired temperature, the first sample was collected and considered as zero time. At zero time, the color loss was less than 5% at the maximum temperature employed in all cases. Then, the voltage was controlled to maintain a constant temperature by applying between 1.0 and 3.0 V. This strategy was followed in order to assess the effect of OH and determine if the application of an electric field exerts any additional effect on the color stability of both studied systems compared with traditional heating.

Figure 1 presents a temperature profile of the conventional and ohmic processed samples at 80 °C. Similar time–temperature profiles were obtained for all conducted treatments (data not shown).

CH process

The CH process was carried out as control, following the methodology previously reported by Morales-Oyervides et al. (2015). Experiments were carried out by using the same OH cell without the application of the electric field. The temperature range was similar to the OH process (60–90 °C). The samples were withdrawn at the same heating time (0–75 min). The

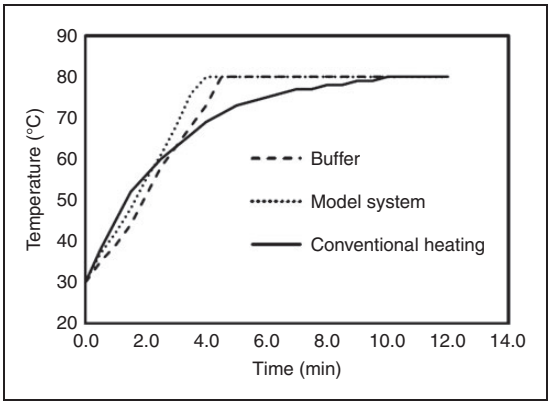

Figure 1. Temperature profiles of *Penicillium purpurogenum* GH2 pigment samples during ohmic and conventional heating treatments at 80 °C.

temperature was monitored using type K thermocouples inserted in the center of the vessel.

Mathematical modeling of color degradation kinetics

First-order kinetic model. Color degradation was fitted to a first-order model (fractional conversion model), considering a fraction of compound (pigment) remaining after a long period of time as previously observed by Morales-Oyervides et al. (2015)

$$OD = OD_{eq} + (OD_0 - OD_{eq})\exp^{-kt} \quad (1)$$

where OD is the optical density at time t, OD_0 *is* the initial value, OD_{eq} is the residual OD (obtained experimentally), and k is the first-order rate constant at temperature T.

The effect of temperature on the rate constant was evaluated using the Arrhenius equation

$$k = k_{ref}.\exp\left[-\frac{Ea}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right] \quad (2)$$

where T_{ref} is the reference temperature (70 °C), k_{ref} (min^{-1}) is the degradation rate at T_{ref}, the E_a is the activation energy (kJ mol^{-1}), R is the ideal gas constant (0.008314 kJmol^{-1} K^{-1}), and T is the temperature (K).

Bigelow's thermal death time (TDT) model. The corresponding expression considering a residual OD after treatment in terms of decimal logarithms and a constant temperature is

$$\log OD = \log(OD_{eq} + \left(OD_0 - OD_{eq}\right)10^{-\frac{t}{D}} \quad (3)$$

where OD is the optical density at time t, and D is the decimal reduction time at the experimental temperature, T.

The effect of temperature on the decimal reduction time can be expressed by

$$\log D_T = \log D_{T_{ref}} 10^{-\frac{T-T_{ref}}{z}} \quad (4)$$

where the value of the decimal reduction time at a reference temperature ($D_{T_{ref}}$) and the TDT parameter (z) are the two parameters of the model.

Bigelow and Arrhenius's models are related according to a straightforward mathematical relationship where E_a and z are related to each other through temperatures T and T_{ref}

$$K_{ref} = \frac{\ln(10)}{D_{ref}}; \quad E_a = \frac{RTT_{ref}\ln(10)}{z} \quad (5)$$

The entire set of data was fitted in the global models by least squares nonlinear regression under conditions of constant temperature obtaining a more precise estimation of the parameters. Thus, it was possible to obtain a better prediction of the behavior of pigments degradation under all the range of temperature evaluated. Based on the above, the global models describing the effect of temperature and time on pigments degradation were described by the following equations

$$OD = OD_0 + (OD_0 - OD_{eq})\exp^{-\left\{k_{ref}.\,exp\left[-\frac{Ea}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right].t\right\}} \quad (6)$$

$$\log OD = \log OD_0 + (OD_0 - OD_{eq})10^{-\frac{10^{\frac{T-T_{ref}}{z}}}{D_{ref}}} \quad (7)$$

The sum of squared residuals by all data fitted in the global models was estimated by

$$SSR = \sum_{k=1}^{n_r}\sum_{i=0}^{n_t}\sum_{j=1}^{n_T}(OD_{r,t,T} - OD_{i,j})^2 \quad (8)$$

where $OD_{r,t,T}$ are the experimental data points and $OD_{i,j}$ are the data points predicted by the model. Models discrimination was based on R^2-values given by $1-SSR/SSD$, where SSD is the sum of squared deviations of data points from the average of the sample.

STATISTICAL ANALYSIS

Three independent experiments were carried out for each temperature and time combinations for pigment

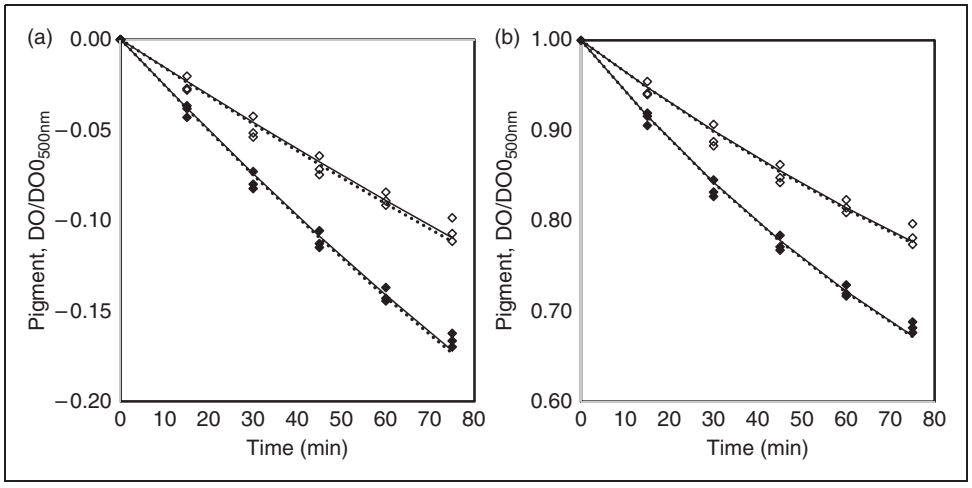

Figure 2. Color degradation kinetics of beverage model system processed by ohmic heating (♦) and conventional heating (◇) at 90 °C described by (a) Bigelow model and (b) first-order model. Full lines correspond to the fit of individual model and the dashed lines to global model.

EX and beverage MS during ohmic and conventional processes. The minimum of the SSRs was determined with the Solver Add-In Microsoft Excel (Microsoft Excel version 2013, Microsoft Corporation). The kinetics parameters were compared using ANOVA and Tukey's test. All the statistical analyses were performed using Statistica 13.0 (Statsoft, Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

Studies conducted in this research showed a high stability of the *P. purpurogenum* GH2 pigment using both heating procedures. Pigment degradation varied around 33 and 23% during OH and conventional thermal processing, respectively, in all conditions analyzed.

Kinetic analysis of color degradation

Thermal stability of *P. purpurogenum* GH2 pigment has been previously investigated by Morales-Oyervides et al. (2015) in a buffer of pH 6. However, the range of temperatures and treatment media used in this investigation for OH were different from those reported by the referred study. Therefore, data in conventional thermal treatment were obtained in order to avoid the necessity of extrapolating the kinetics parameters and being able to compare OH with CH.

Data corresponding to color degradation against time were fitted to the first-order and Bigelow's model so that we could compare and analyze adequately the impact of OH and CH in the color stability of red pigment EX. Both models are the most used approaches for describing the kinetics of quality losses in thermally processed foods (Morales-Oyervides et al., 2015; Ocio et al., 1994; Saraiva et al., 1996). Figure 2 illustrates an example of the fitting of both models to the color degradation curves of the beverage MS processed by OH and CH at 90 °C. These models provided a good fit and proved to be appropriated to describe the color degradation in all the range of experimental conditions investigated. Similar patterns were observed for all the treatments analyzed (data not shown). The equation parameters and the corresponding correlation coefficients describing the influence of temperature on degradation of red pigment are listed in Tables 1 and 2. The time required for achieving the 50% of pigment degradation ($t_{1/2}$) is also presented in Tables 1 and 2. The correlation coefficients obtained were higher or equal to 0.90, indicating that the model used could be applied satisfactorily to describe degradation curves. It can be observed an increment in all rate constants by increasing temperature for both treatments studied.

The first-order rate constant ranged from $(9.0 \pm 1.0) \times 10^{-4}$ to $(8.1 \pm 0.2) \times 10^{-3}\,\mathrm{min}^{-1}$ and from $(9.0 \pm 1.0) \times 10^{-4}$ to $(5.9 \pm 1.0) \times 10^{-3}\,\mathrm{min}^{-1}$ for pigment EX processed by OH and CH, respectively. For beverage MS, the first-order rate constant varied from $(1.1 \pm 0.0) \times 10^{-3}$ to $(8.8 \pm 0.2) \times 10^{-3}\,\mathrm{min}^{-1}$ and from $(9.0 \pm 0.0) \times 10^{-4}$ to $(5.3 \pm 0.2) \times 10^{-3}\,\mathrm{min}^{-1}$ for OH and CH, respectively.

It can be observed from Tables 1 and 2 that heating process type (OH and CH) did not show a statistical difference ($p > 0.05$) in degradation rate constant in the range of 60–70 °C temperature. Conversely, at higher temperature significant differences in the rate constant were observed. For example, at 90 °C the rate constant corresponding to the pigment degradation was around 1.5 times higher for the OH than for the CH independently of the pigmented system. The higher degradation of the pigment by OH as compared with a CH at the

Table 1. Parameters of the first-order-type Arrhenius model for color degradation in the pigment extract during ohmic and conventional heating

Process	T (°C)	k (min^{-1})	D (min)	R^2	$t_{1/2}$ (min)
Conventional heating	60	0.0009 ± 0.0001a	2612.87 ± 224a	0.91	786.55 ± 68a
	70	0.0019 ± 0.0001b	1206.16 ± 42b	0.97	363.09 ± 13b
	80	0.0032 ± 0.0003c	723.36 ± 65b,c,d	0.96	217.75 ± 20b,c,d
	90	0.0059 ± 0.0001e	387.34 ± 5d	1.00	116.60 ± 1d
Ohmic heating	60	0.0009 ± 0.0001a	2151.98 ± 281b	0.96	647.81 ± 85b
	70	0.0020 ± 0.0001b	1123.90 ± 50b,c	0.98	338.33 ± 15b,c
	80	0.0041 ± 0.0001d	562.56 ± 11c,d	0.99	169.35 ± 3c,d
	90	0.0081 ± 0.0002f	284.70 ± 6d	1.00	85.70 ± 2d

Results are the average values of three independents experiments (mean ± standard error of three replicates per sample). Different letters (a–d) indicate significant statistical differences ($p < 0.05$).

Table 2. Parameters of the first-order-type Arrhenius model for color degradation in beverage model system during ohmic and conventional heating

Process	T (°C)	k (min^{-1})	D (min)	R^2	$t_{1/2}$ (min)
Conventional heating	60	0.0009 ± 0.0000a	2518.06 ± 122a	0.90	758.01 ± 37a
	70	0.0016 ± 0.0001a,b	1410.21 ± 107c	0.94	424.51 ± 32c
	80	0.0032 ± 0.0003c	712.13 ± 74d	0.93	214.37 ± 22d
	90	0.0053 ± 0.0002d	431.30 ± 18d,e	0.98	129.83 ± 6d,e
Ohmic heating	60	0.0011 ± 0.0000a	2100.71 ± 49b	0.95	632.38 ± 15b
	70	0.0021 ± 0.0000b	1078.37 ± 23d	0.98	324.62 ± 7d
	80	0.0047 ± 0.0001d	493.85 ± 11d,e	0.99	148.66 ± 3d,e
	90	0.0088 ± 0.0002e	261.10 ± 6e	0.99	78.60 ± 2e

Results are the average values of three independents experiments (mean ± standard error of three replicates per sample). Different letters (a–e) indicate significant statistical differences ($p < 0.05$).

same temperature could be due to a collateral effect caused during OH processing such as the occurrence of electrochemical reactions such as water electrolysis and the electrode corrosion causing electrochemical degradation of pigments by reactions at the electrode (Assiry et al., 2003). A second important effect in the degradation pathways by sample processed under OH could be the polarization phenomena that depend strongly on molecular weight, mobility, and temperature (Icier and Baysal, 2004). When an electric field is applied, the molecules of the sample tend to align with the oscillating electric field producing the phenomenon of polarization, being this dependent on the relaxation time, which is the necessary time to reorganize the random orientation of the molecules' dipoles when an electric field is removed. As it is mentioned above, the differences in the rate constants only appear significant in temperatures higher than 70 °C during OH. Icier and Baysal (2004) have suggested that at high temperatures frequency and relaxation time decrease, thus affecting the polarization process.

The rate constants degradation obtained in the present work permits to illustrate the high thermal stability of *P. purpurogenum* GH2 pigment as compared with other natural pigments treated for OH or CH. Mercali et al. (2013) studied the stability of anthocyanins in acerola pulp in a temperature range of 75–90 °C under OH and CH treatments. They reported that anthocyanins degradation was well described by a first-order reaction model and not significant differences were observed in the rate constants when OH or CH was applied. The degradation rate constant at the highest temperature (90 °C) was between two and three times higher than the values here obtained for *P. purpurogenum* GH2 pigments. Also, Mercali et al. (2015) reported that monomeric anthocyanins degradation of jaboticaba juice was independent of the heating procedure and also followed a first-order degradation pattern. However, the degradation rate of anthocyanins in the juice was similar to the rate values determined in our study by using both technologies. On the other hand, Sarkis et al. (2013) did not observe the difference

between CH and OH during degradation of anthocyanins in blueberry pulp when OH was applied at low voltage gradient. However, as it was observed in the present work, the degradation rate was greater when OH at low levels of voltage was applied at higher temperatures. Other studies indicated that ascorbic acid and carotenoids presented similar degradation rate when OH and CH were compared, suggesting that the presence of oscillating electric field did not affect the degradation mechanism during the OH process (Pez-Jaeschke et al., 2016).

The decimal reduction values (D) and the half-time ($t_{1/2}$) values for each experimental condition evaluated for pigment EX and beverage MS are presented in Tables 1 and 2. For pigment EX, *D-values* and $t_{1/2}$-values ranged from 2152 ± 281 to 285 ± 6 min and between 648 ± 85 and 86 ± 2 min for OH-treated samples, respectively. Meanwhile, *D-values* and $t_{1/2}$-values for CH were found between 2613 ± 224 to 387 ± 5 and 787 ± 68 to 117 ± 1 min, respectively. D-values obtained at 60 and 80 °C were in agreement with those reported by Morales-Oyervides et al. (2015) in a buffer system at the same pH value of 6.0. Also, for the beverage MS D-values and $t_{1/2}$-values ranged from 2101 ± 49 to 261 ± 6 min and between 632 ± 15 and 79 ± 2 min, respectively, for OH, and from 2518 ± 122 to 431 ± 18 min and 758 ± 37 and 130 ± 6 min, for CH, respectively. The observed differences in model parameters between ohmic and conventional treatment could be the result of the effect of the electric field applied under the conditions previously mentioned, considering that these values are directly correlated to the rate of degradation.

The D-values for OH obtained here represent a marked effect of OH on pigments degradation; however, the time required to reduce the number of viable cells by 1 log cycle is much lower for microbial inactivation. Pereira et al. (2007) studied the effect of OH on the death kinetics of *Escherichia coli* in goat milk (55–65 °C, 50 Hz, different field strengths) and *Bacillus licheniformis* in cloudberry jam (70–90 °C, 50 Hz, different field strengths) and reported values of 14.2–0.86 min for *E. coli* and 59.6–1.19 min for *B. licheniformis*. Sun et al. (2008) reported D-values of 6.59–0.16 min for *Streptococcus thermophilus* inactivation in milk under OH processing (70–80 °C, 20 Hz, different field strengths).

In general, red pigments produced by *P. purpurogenum* GH2 presented a very good thermal stability when is subjected to OH technology conditions. In the range of experimental conditions evaluated, *P. purpurogenum* GH2 pigments show a potential for applications in pasteurized food matrices. Treatments at 60 °C for 75 min degraded pigment color less than 5% independently of the heating procedure or treatment

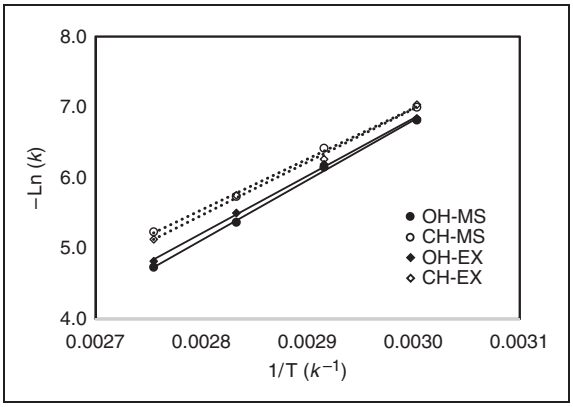

Figure 3. Diagnostic plot of temperature dependence given by the Arrhenius equation during ohmic heating (full lines) and conventional heating (dashed lines).

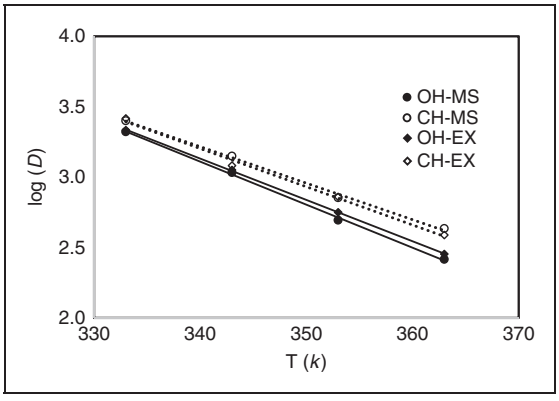

Figure 4. Diagnostic plot of temperature dependence given by the Bigelow equation during ohmic heating (full lines) and conventional heating (dashed lines).

medium. After OH and CH processing at maximum condition evaluated (90 °C for 75 min), the percentage of color degradation for pigment EX was 30 and 24%, respectively.

Modeling temperature dependence of color degradation

Figures 3 and 4 illustrate the effect of temperature on the rate constant k and D_t-values calculated according to equations (2) and (4). These relationships have been widely used to describe the thermal stability of chemical compounds, allowing calculating D_t- and z-values that are essential in the design and optimization of pasteurization processes. The D-value at a reference temperature (D_{ref}) and the z-value are the basis of TDT method, which is the current procedure in industrial practice for most pasteurization and sterilization processes.

Table 3. Kinetic parameters of global models for pigments degradation during ohmic and conventional heating

	First-order Arrhenius type model			Bigelow TDT model		
Process	E_a (kJ mol^{-1})	k_{ref} (min^{-1})	R^2	D_{ref} (min)	z (°C)	R^2
	Pigment extract			Pigment extract		
Conventional	61.878 ± 3.30a	0.002 ± 0.00013a	0.99	958.58 ± 56.24a	38.19 ± 1.88a	1.00
Ohmic	70.318 ± 0.48a	0.003 ± 0.00003b	1.00	791.29 ± 15.34b	33.80 ± 0.40a	1.00
	Beverage model system			Beverage model system		
Conventional	59.066 ± 1.52a	0.002 ± 0.00016a	0.98	999.01 ± 75.00a	40.11 ± 0.98a	1.00
Ohmic	71.036 ± 1.78b	0.003 ± 0.00003b	0.99	725.98 ± 18.55a	33.46 ± 0.84b	1.00

TDT: thermal death time.
Results are the average values of three independents experiments (mean ± standard error of three replicates per sample).
Different letters (a and b) indicate significant statistical differences ($p < 0.05$).

Table 3 presents the parameters obtained from equations (6) and (7). Both models provided a good fit to the rate constants k and D_t-values and allowed to calculate the activation energy (E_a) and the thermal resistance coefficient (z-value) for both treatments and media tested. The E_a corresponding to the thermal degradation of *P. purpurogenum* GH2 pigment EX for both heating procedures was in the range of E_a previously reported for thermal inactivation of the same pigment or anthocyanins (Mercali et al., 2013, 2015; Morales-Oyervides et al., 2015).

Statistical comparison of this parameter was performed separately for the media treatment studied (Tukey's test). Only the evaluated treatments in the beverage MS showed significant differences ($p < 0.05$), E_a-value for OH was higher (71.04 ± 1.78 kJmol^{-1}) in comparison with the value obtained by CH (59.07 ± 1.52 kJmol^{-1}).

On the other hand, no statistical differences ($p > 0.05$) were observed between the E_a-values corresponding to the pigment EX processed by OH and CH processes.

However, the z-values for the pigmented EX processed under OH and CH were 33.80 ± 0.40 and 38.19 ± 1.88 °C, respectively. In reference to beverage MS, the z-values for OH and CH were 33.46 ± 0.84 and 40.11 ± 0.98 °C, respectively.

The z-values obtained here for both treatments are significantly higher than other natural pigments like carotenoids present in orange juice (Fratianni et al., 2010) and citrus juice (Dhuique-Mayer et al., 2007) with z-values ranged between 10.2 and 22.5 °C. Not to mention that are higher than those for microorganisms/enzyme inactivation. Onwnka et al. (2008) reported z-values of 4 °C for *E. coli* and 20 °C for *Salmonella* spp., presenting higher sensitive temperature than pigments here evaluated. Comparable values were reported by Aghajanzadeh et al. (2016) obtaining a z-value of 36.90 °C for pectin methylesterase in sour orange juice during heat treatment. Even though the obtained z-values are higher with CH than with OH, the difference between both heating treatments is much lower than that observed in microbial inactivation (Pereira et al., 2007). Studies have reported that OH may present nonthermal cellular damage due to the electric field. Therefore, a considerable reduction of z-value is observed for microbial inactivation under OH when compared to CH methods (Pereira et al., 2007; Sun et al., 2008).

Moreover, experiments demonstrated that the higher sensitivity of the pigment to temperature leads to high temperature and short time (HTST) conditions being ideal. Ohmic processing enables heating at a high rapid rate and thus makes possible to achieve HTST conditions; these conditions are hardly achieved using CH meaning that process optimization with OH technology should permit even greater retention of the color intensity.

Thermodynamic analysis

The good fit of kinetic parameters provided by the first-order-type Arrhenius model enabled the calculation of apparent thermodynamic parameters that allowed to infer about structure and stability aspects during the degradation processes of *P. purpurogenum* GH2 pigments.

The change of activation enthalpy ($\Delta H^{\neq}$), free energy of inactivation ($\Delta G^{\neq}$), and activation entropy ($\Delta S^{\neq}$) for pigments degradation in the different systems were calculated according to

$$\Delta H^{\neq} = E_a - R.T \tag{9}$$

$$\Delta G^{\neq} = -R.T.ln\left(\frac{k.h}{k_B.T}\right) \tag{10}$$

$$\Delta S^{\neq} = \frac{\Delta H^{\neq} - \Delta G^{\neq}}{T} \quad (11)$$

where h is the Planck's constant (3.3232×10^{-34} J s) and K_B is the Boltzmann constant (1.3806×10^{-23} J K^{-1}). Values of thermodynamic parameters are listed in Table 4.

$\Delta H^{\neq}$ values provide a measure of the energy difference between the reagent and active complex representing the energy barrier that must be overcome by the molecules during the reaction. This parameter is related to the number of noncovalent bonds broken resulting in a structure alteration in the system (Bhatti et al., 2006; Georgieva et al., 2012).

In this study, $\Delta H^{\neq}$ values for the pigment EX and beverage MS were statistically different between the heating processes. $\Delta H^{\neq}$ values for pigmented EX varied from 67.30 ± 0.50 to 67.55 ± 0.50 kJmol^{-1} and from 58.86 ± 3.30 to 59.11 ± 0.50 kJmol^{-1} for OH and CH treatments, respectively. Similarly, $\Delta H^{\neq}$ values for beverage MS ranged between 68.02 ± 1.80 and 68.27 ± 1.80 kJmol^{-1} and from 56.05 ± 3.30 to 56.30 ± 1.59 kJmol^{-1} for OH and CH treatments, respectively. OH processed samples showed higher values of $\Delta H^{\neq}$ indicating a greater structure alteration due to the electric field applied. Contrary to above described, several authors did not observe significant differences in $\Delta H^{\neq}$ values in anthocyanins treated by OH and CH in acerola pulp and jaboticaba juice (Mercali et al., 2013, 2015). The positive sign of $\Delta H^{\neq}$ evidence that two evaluated systems presented an endothermic reaction with heat adsorption. Also, it can be observed that $\Delta H^{\neq}$ increased with temperature in both systems, indicating that the pigment conformation could be modified (Bhatti et al., 2006; Morales-Oyervides et al., 2015).

$\Delta G^{\neq}$ is a criterion of equilibrium and spontaneity; in all cases for both pigment EX and beverage model processed under OH and CH $\Delta G^{\neq}$ presented positive values, which means that *P. purpurogenum* GH2 pigments degradation is a nonspontaneous reaction, presenting a resistance against thermal unfolding. Independently of the technology applied and system evaluated, pigments presented similar values oscillating between 100.82 and 105.26 kJmol^{-1}, being this value close to those reported by several authors for natural pigments (Mercali et al., 2013, 2015; Morales-Oyervides et al., 2015; Terra-Silveira et al., 2013).

Finally, $\Delta S^{\neq}$ measures the disorder change of the system to a molecular level and negative entropy values suggested that there were negligible disorders of the molecules. $\Delta S^{\neq}$ values for pigment EX ranged between -126.58 and -127.30 mol^{-1} K^{-1} and from -100.52 to 101.24 mol^{-1} K^{-1} for CH and OH, respectively. Instead, $\Delta S^{\neq}$ values in beverage MS were in the range of -134.85 to 135.57 and from -97.74 to -98.46 mol^{-1} K^{-1} for CH and OH, respectively. Nevertheless, according to the negative values obtained in all cases a low level of disorderliness in the system during the degradation process (Bhatti et al., 2006).

Table 4. Thermodynamic parameters obtained for pigments degradation during ohmic and conventional heating

Process	T (K)	$\Delta H^{\neq}$ (kJ mol^{-1})	$\Delta G^{\neq}$ (kJ mol^{-1})	$\Delta S^{\neq}$ (J mol^{-1})
Pigment extract				
Conventional	333	59.11a	101.26a	−126.58a
	343	59.03a	102.53b,c	−126.83a
	353	58.94a	103.80d,e	−127.07a
	363	58.86a	105.07e	−127.30a
Ohmic	333	67.55a	101.02a	−100.52a
	343	67.47a	102.03b	−100.77a
	353	67.38a	103.04c,d	−101.01a
	363	67.30a	104.05f	−101.24a
Model system				
Conventional	333	56.30a	101.20a,b	−134.85a
	343	56.21a	102.55c	−135.10a
	353	56.13a	103.90d	−135.33a
	363	56.05a	105.26e	−135.57a
Ohmic	333	68.27b	100.82b	−97.74b
	343	68.18b	101.79b	−97.99b
	353	68.10b	102.78c	−98.23b
	363	68.02b	103.76d	−98.46b

Results are the average values of three independents experiments (mean ± standard error of three replicates per sample).
Different letters (a–d) indicate significant statistical differences ($p < 0.05$).

CONCLUSION

Pigment degradation kinetics of red pigments produced by *P. purpurogenum* GH2 under ohmic and CH treatment was well described by the Bigelow and Arrhenius models ($R^2 = 0.98$–1.00). Red pigments showed good stability (degradation varying from less than 1.0% to around 32.8%, depending on processing conditions). No significant difference was obtained when the pigment EX or the model beverage was submitted to both heating treatments.

OH treatment showed a higher effect on the pigments than CH treatment; however, this effect is less pronounced than that observed for microbial inactivation. At optimum microbial inactivation conditions with OH treatment, degradation can be negligible depending on the target microorganism.

Fungal pigments showed a very good stability under both CH and OH, showing promising results for future applications in heat pasteurized food systems. Nonetheless, red pigments produced by *P. purpurogenum* GH2 are potentially useful for products pasteurized under HTST conditions, which can be easily achieved by OH. Process optimization with this technology will allow achieving higher color retention.

RESEARCH HIGHLIGHTS

- Fungal pigments presented high stability under ohmic heating technology.
- Degradation kinetics during ohmic and conventional heating processing were well described by first-order and Bigelow models.
- Nonthermal effects could influence the color degradation rate in fungal pigments' extracts and beverage model systems.

DECLARATION OF CONFLICTING INTERESTS

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REFERENCES

Aberoumand A. (2005). A review article on edible pigments properties and sources as natural biocolorants in foodstuff and food industry. *World Journal of Dairy and Food Sciences* 6(22): 71–78.

Achir N, Dhuique-Mayer C, Hadjal T, Madani K, Pain J and Dornier M. (2016). Pasteurization of citrus juices with ohmic heating to preserve the carotenoid profile. *Innovative Food Science and Emerging Technologies* 33: 397–404.

Aghajanzadeh S, Mohammad A, Kashaninejad M, Maghsoudlou Y and Esmailzadeh E. (2016). Thermal inactivation kinetic of pectin methylesterase and cloud stability in sour orange juice. *Journal of Food Engineering* 185: 72–77.

Assiry A, Sastry SK and Samaranayake C. (2003). Degradation kinetics of ascorbic acid during ohmic heating with stainless steel electrodes. *Journal of Applied Electrochemistry* 33(2): 187–196.

Bansal B and Chen XD. (2006). Effect of temperature and power frequency on milk fouling in an ohmic heater. *Food and Bioproducts Processing* 84(4): 286–291.

Bhatti HN, Asgher M, Abbas A, Nawaz R and Sheikh MA. (2006). Studies on kinetics and thermostability of a novel acid invertase from *Fusarium solani*. *Journal of Agricultural and Food Chemistry* 54(13): 4617–4623.

Bozkurt H and Icier F. (2012). The change of apparent viscosity of liquid whole egg during ohmic and conventional heating. *Journal of Food Process Engineering* 35(1): 120–133.

De Alwis A and Fryer PJ. (1992). Operability of the ohmic heating process: Electrical conductivity effects. *Journal of Food Engineering* 15(1): 21–48.

De Carvalho JC. (2004). *Desenvolvimento de bioprocesso para a produção de pigmentos a partir de Monascus por fermentação em substrato sólido*. PhD Thesis. Universidade Federal do Paraná, Curitiba, Brasil.

Dhuique-Mayer C, Tbatou M, Carail M, Caris-Veyrat C, Dornier M and Josephe M. (2007). Thermal degradation of antioxidant micronutrients in *Citrus* juice: Kinetics and newly formed compounds. *Journal of Agriculture and Food Chemistry* 55(10): 4209–4216.

Dufossé L. (2006). Microbial production of food grade pigments. *Food Technology and Biotechnology* 44(3): 313–321.

Dufossé L, Fouillaud M, Caro Y, Mapari SA and Sutthiwong N. (2014). Filamentous fungi are large-scale producers of pigments and colorants for the food industry. *Current Opinion in Biotechnology* 26: 56–61.

Dyrby M, Westergaard N and Stapelfeldt H. (2001). Light and heat sensitivity of red cabbage extract in soft drink model systems. *Food Chemistry* 72(4): 431–437.

Fernández-López J, Angosto JM, Giménez PJ and León G. (2013). Thermal stability of selected natural red extracts used as food colorants. *Plant Foods for Human Nutrition* 68(1): 11–17.

Fratianni A, Cinquanta L and Panfili G. (2010). Degradation of carotenoids in orange juice during microwave heating. *LWT—Food Science and Technology* 43(6): 867–871.

Georgieva V, Zvezdova D and Vlaev L. (2012). Non-isothermal kinetics of thermal degradation of chitosan. *Chemistry Central Journal* 6(1): 11–17.

Guida V, Ferrari G, Pataro G, Chambery A, Di Maro A and Parente A. (2013). The effects of ohmic and conventional blanching on the nutritional, bioactive compounds and quality parameters of artichoke heads. *LWT—Food Science and Technology* 53(2): 569–579.

Icier F and Baysal T. (2004). Dielectrical properties of food materials-2: Measurement techniques. *Critical Reviews in Food Science and Nutrition* 44(6): 473–478.

Lee J, Kim S and Kang D. (2015). Effect of pH for inactivation of *Escherichia coli* O157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* in orange juice by ohmic heating. *LWT—Food Science and Technology* 62(1): 83–88.

Mapari SA, Hansen ME, Meyer AS and Thrane U. (2008). Computerized screening for novel producers of Monascus-like food pigments in *Penicillium* species. *Journal of Agricultural and Food Chemistry* 56(21): 9981–9989.

Mapari SA, Meyer AS, Thrane U and Frisvad JC. (2009). Identification of potentially safe promising fungal cell factories for the production of polyketide natural food colorants using chemotaxonomic rationale. *Microbial Cell Factories* 8(1): 1.

Mapari SA, Nielsen KF, Larsen TO, Frisvad JC, Meyer AS and Thrane U. (2005). Exploring fungal biodiversity for

the production of water-soluble pigments as potential natural food colorants. *Current Opinion in Biotechnology* 16(2): 231–238.

Mapari SA, Thrane U and Meyer AS. (2010). Fungal polyketide azaphilone pigments as future natural food colorants? *Trends in Biotechnology* 28(6): 300–307.

Méndez-Zavala A. (2011). *Identificación de factores que afectan la producción de pigmentos por Penicillium purpurogenum GH2 y obtención de perfiles cromatográficos*. Masters Thesis, Universidad Autónoma de Coahuila, Saltillo, Coahuila, México.

Mercali GD, Gurak PD, Schmitz F and Marczak LD. (2015). Evaluation of non-thermal effects of electricity on anthocyanin degradation during ohmic heating of jaboticaba (*Myrciaria cauliflora*) juice. *Food Chemistry* 171: 200–205.

Mercali GD, Jaeschke DP, Tessaro IC and Marczak LD. (2013). Degradation kinetics of anthocyanins in acerola pulp: Comparison between ohmic and conventional heat treatment. *Food Chemistry* 136(2): 853–857.

Morales-Oyervides L, Oliveira JC, Sousa-Gallagher MJ, Méndez-Zavala A and Montañez JC. (2015). Effect of heat exposure on the colour intensity of red pigments produced by *Penicillium purpurogenum* GH2. *Journal of Food Engineering* 164: 21–29.

Ocio MJ, Fernandez PS, Alvarruiz A and Martinez A. (1994). Comparison of TDT and Arrhenius models for rate constant inactivation predictions of *Bacillus stearothermophilus* heated in mushroom-alginate substrate. *Letters in Applied Microbiology* 19(2): 114–117.

Onwnka UN, Akobundu ENT and Iwe MO (2008). Kinetics of inactivation of Listeria monocytogen, Clostridium perfringens, Escherichia coli and Salmonella spp. in ohmic heated tomato juices. *Journal of Pure Applied Microbiology* 2: 29–38.

Pereira R, Martins J, Mateus C, Texeira JA and Vicente AA. (2007). Death kinetics of *Escherichia coli* in goat milk and *Bacillus licheniformis* in cloudberry jam treated by ohmic heating. *Chemical Papers* 61(2): 121–126.

Pez-Jaeschke D, Ferreira-Marczak L and Domeneghini-Mercali G. (2016). Evaluation of non-thermal effects of electricity on ascorbic acid and carotenoid degradation in acerola pulp during ohmic heating. *Food Chemistry* 199: 128–134.

Rawson A, Patras A, Tiwari BK, Noci F, Koutchma T and Brunton N. (2011). Effect of thermal and non thermal processing technologies on the bioactive content of exotic fruits and their products: Review of recent advances. *Food Research International* 44(7): 1875–1887.

Samaranayake CP and Sastry SK. (2016a). Effect of moderate electric fields on inactivation kinetics of pectin methylesterase in tomatoes: The roles of electric field strength and temperature. *Journal of Food Engineering* 186: 17–26.

Samaranayake CP and Sastry SK. (2016b). Effects of controlled-frequency moderate electric fields on pectin methylesterase and polygalacturonase activities in tomato homogenate. *Food Chemistry* 199: 265–272.

Saraiva J, Oliveira JC, Hendrickx M, Oliveira FA and Tobback P. (1996). Analysis of the inactivation kinetics of freeze-dried α-amylase from *Bacillus amyloliquefaciens* at different moisture contents. *Lebensmittel-Wissenschaft und -Technologie* 29: 260–266.

Sarkis JR, Jaeschke DP, Tessaro IC and Marczak LD. (2013). Effects of ohmic and conventional heating on anthocyanin degradation during the processing of blueberry pulp. *LWT—Food Science and Technology* 51(1): 79–85.

Silveira ST, Daroit DJ, Sant Anna V and Brandelli A. (2013). Stability modeling of red pigments produced by Monascus purpureus in submerged cultivations with sugarcane bagasse. *Food and Bioprocess Technology* 6(4): 1007–1014.

Stirling R. (1987). Ohmic heating—A new process for the food industry. *Power Engineering Journal* 1(6): 365–371.

Sun H, Kawamura S, Himoto J, Itoh K, Wada T and Kimura T. (2008). Effects of ohmic heating on microbial counts and denaturation of proteins in milk. *Food Science and Technology Research* 14(2): 117–123.

Terra-Silveira S, Daroit DJ, Sant'Anna V and Brandelli A. (2013). Stability modeling of red pigments produced by *Monascus purpureus* in submerged cultivations with sugarcane bagasse. *Food and Bioprocess Technology* 6(4): 1007–1014.

Vendruscolo F, Luise-Müller B, Esteves-Moritz D, De Oliveira D, Schmidell W and Luiz-Ninow J. (2013). Thermal stability of natural pigments produced by *Monascus ruber* in submerged fermentation. *Biocatalysis and Agricultural Biotechnology* 2(3): 278–284.