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Biphasic short time heat degradation of the blue microalgae protein phycocyanin from *Arthrospira platensis*

L. Böcker[a], S. Ortmann[a], J. Surber[a], E. Leeb[b], K. Reineke[b], A. Mathys[a,*]

[a] ETH Zurich, Institute of Food, Nutrition and Health IFNH, Sustainable Food Processing Laboratory, Switzerland
[b] GNT Europa GmbH, Germany

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ABSTRACT

Stability of colorants is concerning for food coloring matrices, particularly for the only natural blue food coloring, phycocyanin. The *Spirulina*-based microalgal extract is mainly comprised of heat sensitive protein-chromophore complexes, C-phycocyanin and allophycocyanin. Although frequently encountered in food processing, the impact of short time heat treatments has not been studied systematically. Here, phosphate buffered phycocyanin solution was heated in batch and emerging continuous processing systems, both characterized with high surface-to-volume ratios allowing isothermal conditions with residence times down to 5 s. Absorption scans revealed biphasic degradation of phycocyanin color activity to about 30% within 30 s at $T \geq 70\,^{\circ}C$. Kinetic modelling of the color decay via an nth order approach contradicts previously assumed linear first order kinetics with a best fitting empirical reaction order of $n = 6$. It shows that decay in phycocyanin color activity is not a single process but encompasses C-phycocyanin and allophycocyanin aggregate disintegration and denaturation. *Industrial relevance:* Central to this study is the color stability of phycocyanin, which is a high value component, derived from the emerging food source microalgae. It is also the only naturally obtained blue food coloring available to the food industry. Insights could be gained on the color degradation kinetics by treating an industry relevant formulation in batch and emerging scalable continuous systems via micro process engineering. This data will directly support food research and development activities to optimize and minimize blue color losses within multiple product categories.

1. Introduction

Spirulina Blue is the only naturally derived blue food coloring that is available to the food industry so far. After a 2007 study in Great Britain revealed that artificial colorants have an impact on hyperactivity in children (McCann et al., 2007), as well as the focus on clean labelling, non-artificial colorants have received increased attention. Phycocyanins are protein chromophore complexes in light harvesting complexes of different organisms, prominently in *Arthrospira platensis*. The cyanobacterium *A. platensis*, commonly known as *Spirulina*, is categorized as a microalga (Pulz & Gross, 2004; Vonshak, 1997). It is photoautotrophic and is used as a nutraceutical, food ingredient, or food coloring. The main phycocyanin fractions are C-phycocyanin (cPC) and allophycocyanin (aPC) with maximum absorbance at 620 nm and 650 nm, respectively. Both phycobiliproteins are composed of two apoprotein chains as backbone to which three or two phycocyanobilins are covalently bound via thioether bonds in case of cPC or aPC, respectively (MacColl, 1998). Due to their fluorescence and distinct visible absorption, phycobiliproteins have been subjected to multiple analytical tools as model proteins for studying protein properties (Berns & MacColl, 1989). Denaturation of the secondary, tertiary, or quaternary structure of the apoprotein results in a rearrangement of the linear chromophore into a cyclic form with a subsequent absorbance increase at 360 nm (MacColl, 1998).

Stability of phycocyanin in food or food formulations and during processing is of interest to the entire food industry as it is a limiting factor in its application (Buchweitz, 2016). Besides its instability in acidic conditions, color activity is also lost due to thermal processing (Jespersen, Strømdahl, & Olsen, 2005; Martelli, Folli, Visai, Daglia, & Ferrari, 2014). Researchers dealt with this topic and proposed encapsulation techniques or stabilizing effects through the addition of sodium dodecyl sulfate (SDS) (Falkeborg, Roda-Serrat, Burnæs, & Nielsen, 2018) or of sugars (Chaiklahan, Chirasuwan, & Bunnag, 2012; Martelli et al., 2014). However, the kinetics of color degradation have not been extensively studied. High temperature short time (HTST) effects, in particular, which are abundantly found in food processing,

* Corresponding author.
E-mail address: alexander.mathys@hest.ethz.ch (A. Mathys).

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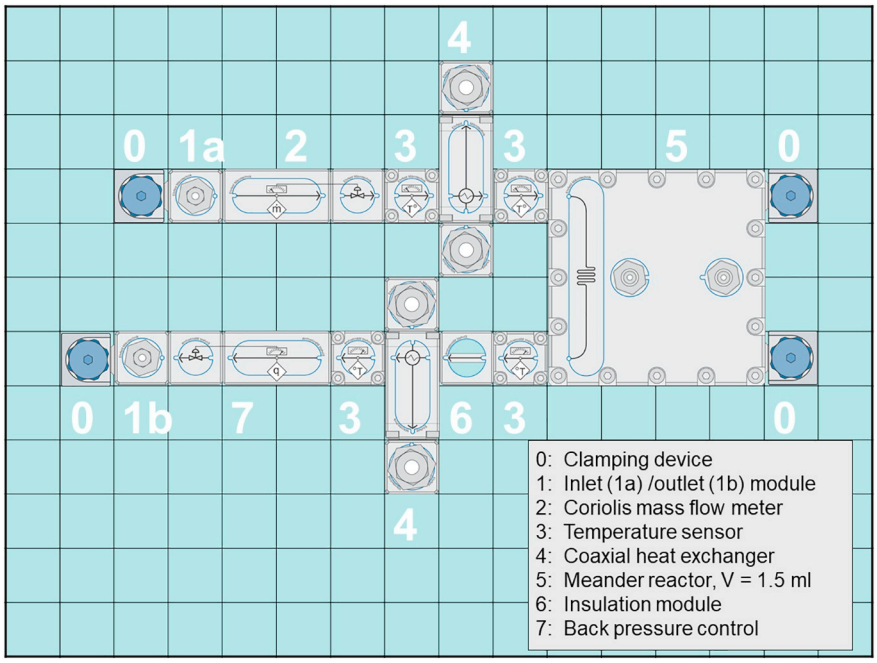

Fig. 1. Scheme of the modular micro reaction system (MMRS). Sample solution was fed into the system via the inlet (1a). It was pumped via the Coriolis mass flow meter (2) through a coaxial heat exchanger (4). Temperature was kept in the meander reactor (5) before the sample was cooled down in the second coaxial heat exchanger (4) and left the system via the outlet module (1b) (Graphics adapted from Ehrfeld Mikrotechnik GmbH, Germany).

have not been fully explored. Focus of other studies was often on lower temperatures and longer time scales (Antelo, Costa, & Kalil, 2008; Chaiklahan et al., 2012; Jespersen et al., 2005; Patel, Pawar, Mishra, Sonawane, & Ghosh, 2004). Yet, prominent examples for application of *Spirulina* extracts imply a HTST process such as dairy products which are commonly pasteurized at 72–75 °C for 15 to 30 s (IDFA - International dairy foods association, 2018; Kessler, 2002). Innovative treatments including pulsed electric fields (PEF) or high pressure (HP) treatments could be considered to replace HTST pasteurization. Though currently, these emerging technologies still face some limitations in scalability and economic conditions, such as investments, in comparison to widely used thermal processing. Moreover, depending on the applied conditions, they also involve a short time temperature increase, e.g. PEF based pasteurization due to ohmic heating and HP due to adiabatic heating (Sevenich & Mathys, 2018; Toepfl, Mathys, Heinz, & Knorr, 2006).

Controlled short heating trials require fast heating and cooling in order to not affect the processing time. Heating and cooling are determined by volumetric heat transfer capabilities as can be derived from the description of residence time in continuous heat transfer processes (Mathys, 2018). Thus, extremely short processing times can be achieved with high surface-to-volume ratios. Consequently, this study applied indirect heating with batch and continuous systems with high surface-to-volume ratios to elucidate thermal color degradation kinetics of a phycocyanin formulation at times below 1 min, typical for continuous food processing. After thermal processing, absorption scans of the samples were taken with a spectrophotometer and the obtained data were modelled with an n^{th} order approach to determine the reaction order for the degradation kinetics.

2. Materials & methods

The current investigation looked at short time thermal degradation of the blue microalgae protein phycocyanin and modelled the degradation kinetics to improve its application in food systems.

2.1. Phycocyanin solution

A spray dried blue powder formulation of the microalga *Arthrospira platensis* was provided by GNT International B.V. (Mierlo, Netherlands). Spray drying of the *Spirulina* extract was performed with 56 g maltodextrin as carrier material per 100 g final dried powder. The powder with 15.7% (w/w) phycocyanin was dissolved at 1 g L^{-1} in phosphate buffer (0.1 M, pH 7.3) containing NaN_3 (10^{-3} M). cPC and aPC were present in a ratio of 71 to 29 in the phycocyanin fraction. Phosphate buffer was chosen due to its pK_a and therefore pH stability independent of temperature (Reineke, Mathys, & Knorr, 2011).

2.2. Thermal treatment in batch and continuous process

For the batch treatment, glass capillaries from Kleinfeld Labortechnik GmbH (Gehrden, Germany) were used with 0.9 mm inner diameter, 1.3 mm outer diameter, and 200 mm length, described elsewhere (Mathys, Heinz, Schwartz, & Knorr, 2007). Capillaries were thermally sealed. Thus, they were only filled with 150 µl phycocyanin solution and not fully to omit thermal treatment of the protein solution during sealing. Once sealed, the capillaries were heated in an oil bath (JULABO GmbH, Seelbach, Germany) at 70, 75 or 80 °C for 5 to 60 s and consecutively transferred into an ice-bath after the specified heating times. The surface-to-volume ratio was 4444 m^{-1}. Numerical simulations showed isothermal conditions after ca. 3 s within the oil bath (Mathys et al., 2007).

For continuous processing, phycocyanin solution was processed through a modular micro reaction system (MMRS), described elsewhere (Georget, Sauvageat, Burbidge, & Mathys, 2013; Mathys, 2018), following the configuration depicted in Fig. 1. The MMRS (Ehrfeld Mikrotechnik GmbH, Germany) was set up to transport samples by a gear pump (HNP Mikrosysteme GmbH, Schwerin, Germany), heat the sample by a first coaxial heat exchanger to 70, 75 or 80 °C, keep the temperature constant in a tailor-made meander reactor of 1.5 ml inner volume, and cool it down in a second coaxial heat exchanger (Ehrfeld Mikrotechnik GmbH, Germany). For retention volume 2.215 ml were considered including temperature sensors (3) and parts of the coaxial heat exchangers (4). The surface-to-volume ratio of the meander reactor was 2610 m^{-1} (Georget et al., 2013). Temperature was tracked online with temperature sensors before and after both coaxial heat exchangers by measuring the sample temperature in the center of the tube (Ehrfeld Mikrotechnik GmbH, Germany). Solution's density was measured to $\rho = 1.005 \pm 0.005$ g/ml with the Coriolis mass flow meter (Bronkhorst AG, Reinach, Switzerland). Mass flow rates were set accordingly

between 2.22 and 26.58 g/min to control residence times from 5 to 60 s. System control was done with TestMB (LabVision 2.9, HiTec Zang GmbH, Germany) allowing isothermal treatment with controlled residence times. Cleaning in place (CIP) was conducted before every trial with deionized water, acid (P3-horolith CIP, Ecolab GmbH, Reinach, Switzerland) and base (Mip SC, Ecolab GmbH, Reinach, Switzerland) solution.

2.3. Phycocyanin concentration

Absorption spectra of native and heat-treated phycocyanin solutions were measured without any further dilution in triplicates from 800 to 300 nm with a UV Vis spectrophotometer (Cary 100, Agilent Technology, US). Absorption values were corrected with the absorption at 800 nm to account for the absorption of colloidal particles. Alternatively, preliminary tests were run to eliminate colloidal particles after processing by centrifugation. Pellets in these tests were blue, indicating that relevant chromophores would have been discarded from the spectrophotometric measurement. By applying the spectrophotometric validated correlation from Yoshikawa and Belay (2008), cPC and aPC concentrations could be derived for each temperature-time trial:

$$cPC = 0.162 \cdot A_{620} - 0.098 \cdot A_{650} \quad (1)$$

$$aPC = 0.180 \cdot A_{650} - 0.042 \cdot A_{620} \quad (2)$$

herein, *cPC* is the concentration of C-phycocyanin ($g\,L^{-1}$), *aPC* is the concentration of allophycocyanin ($g\,L^{-1}$), A_{620} is the absorption at 620 nm and A_{650} is the absorption at 650 nm. The sum of cPC and aPC is the total color active phycocyanin (C_t).

2.4. Kinetic modelling

The degradation of color active phycocyanin was modelled via an nth order approach (Eq. (3)) with TableCurve 2D (Versions 5.01, Systat Software Inc., US).

$$\frac{C_t}{C_0} = [1 + (n-1)\cdot k\cdot t]^{\frac{1}{1-n}} \quad with\ k = k_n \cdot C_0^{n-1} \quad (3)$$

herein C_t is the color active phycocyanin concentration at time t (s), C_0 is the color active phycocyanin concentration at $t = 0\ s$, n is the reaction order, k is the reaction rate constant (s^{-1}), k_n is the specific reaction rate constant and t is the processing time (s) during isothermal conditions. Fitted values for the degradation were obtained by varying the reaction order n and fitting the reaction rate constant k, also described elsewhere (Mathys, 2008). Thereof, the best describing empirical reaction order n was identified through the minimal cumulative standard error of all fitted values.

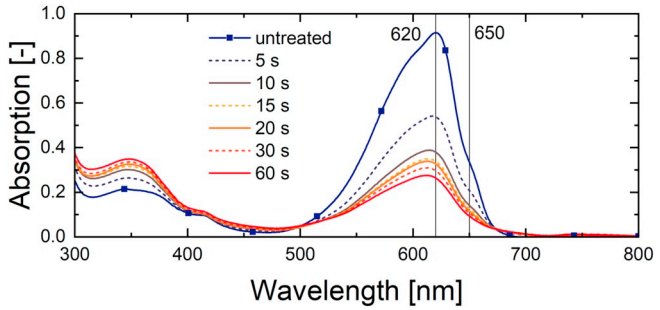

Fig. 2. Absorption spectra of phycocyanin solution 1 $g\,L^{-1}$ in 0.1 M phosphate buffer at pH 7.3, thermally treated for 5–60 s at 70 °C in comparison to an untreated control.

2.5. Energy of activation

With the reaction rate constant k(T) for the best fitting reaction order, the apparent energy of activation (E_a) was determined following the Arrhenius (1889) equation (Eq. (4)),

$$\ln k = \ln k_0 - \frac{E_a}{R}\cdot\frac{1}{T} \quad (4)$$

herein k is the reaction rate constant, k_0 is the reaction rate constant at $T \rightarrow 0\ K$, E_a is the energy of activation ($J\,mol^{-1}$), R is the universal gas constant with 8.314 $J\,mol^{-1}\,K^{-1}$ and T is the absolute temperature (K). k as the effective reaction rate constant was taken for the energy of activation calculations in Eq. (4) instead of the specific reaction rate constant k_n since ln k = ln k_n + (n − 1) ln C_0 (Eq. (3)).

3. Results & discussion

The thermal degradation kinetics of phycocyanin in short time heat processes were central to this study. Fig. 2 depicts by way of example absorption spectra of untreated and thermally processed phycocyanin solutions at 70 °C. The untreated phycocyanin solution had a main peak with an absorbance maximum at 620 nm, with a shoulder at 650 nm and a minor local maximum at 360 nm. These three wavelengths correlate with the peak absorbance of cPC (620 nm), aPC (650 nm), and denatured cPC (360 nm) (MacColl, Csatorday, Berns, & Traeger, 1981). Upon increasing processing time up to 60 s, the absorption peaks at 620 nm and 650 nm decreased, while the 360 nm peak increased. By applying Eqs. (1) and (2), the decay of color activity could be quantified. The experimentally established formulas translate the absorption at 620 and 650 nm into concentrations of cPC and aPC based on a calibration with purified cPC and aPC in phosphate buffer (0.1 M) at pH 6.0 (Yoshikawa & Belay, 2008). Hence, the reported values reflect an equivalent amount of phycocyanin under these conditions and resemble a measure for overall color activity of the phycocyanin solution. The concentrations are referred to as color active phycocyanin concentration since the actual phycocyanin concentration did not change due to thermal processing. Loss in color activity was based on either changes in aggregation state of the biliproteins and/or due to conformational changes of the phycocyanobilin chromophores (Berns & MacColl, 1989), which is discussed in more detail in the modelling Section (3.2).

3.1. Short time thermal degradation of phycocyanin

Relative concentrations of color active phycocyanin are displayed in logarithmic values over processing time in Fig. 3. For both batch and continuous processing, the data show a biphasic decay over treatment time for temperatures T ≥ 70 °C. Color activity dropped within 30 s to about 30% of the initial value and tailed with increasing treatment time. Both processing modes, batch and continuous, were investigated in this study as they encompass distinctly different thermal processing conditions. Heating in the applied batch operation is limited to mainly conductive heat exchange whereas in the continuous system heating is a combination of conduction and convection.

The findings are in agreement with results reported on the degradation of purified cPC by Scheer and Kufer (1977). UV Vis measurements of thermally processed cPC solution at 71 °C for 1 min revealed reductions to 30% of absorbance at 620 nm. However, other authors found much longer decay kinetics, such as Chaiklahan et al. (2012) with a half-life value of 6 min for the degradation of phycocyanin in a citrate phosphate buffer at pH 7.0 and 69 °C.

Discrepancies might be linked to different processing parameters and differences in the phycocyanin sources. This study investigated a food coloring formula containing 15.7% (w/w) phycocyanin with a cPC to aPC ratio of 71 to 29. It is a formulation with a high concentration of phycocyanin and relevant for applications in the food industry,

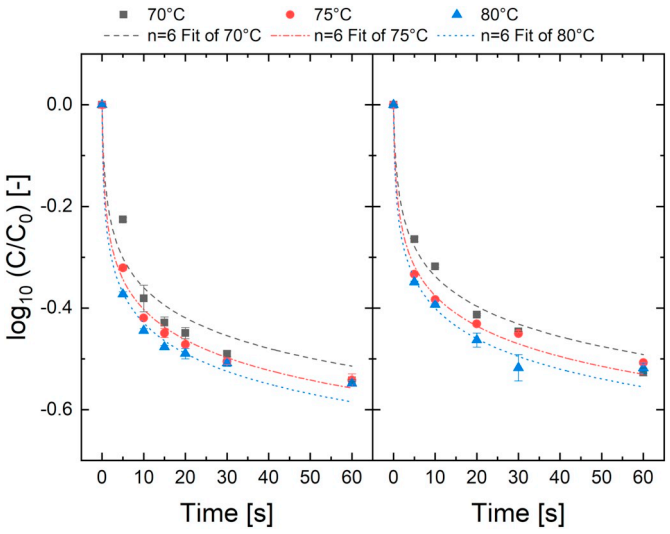

Fig. 3. Relative color active phycocyanin concentration plotted in logarithmic values over processing time and fitting curves with reaction order n = 6. A – batch processing in glass capillaries. B – continuous processing in modular micro reaction system (MMRS). RMSE of the fits for processing temperatures of 70, 75, and 80 °C were 0.045, 0.014, and 0.012 in batch processing and 0.017, 0.012, and 0.013 in continuous processing, respectively.

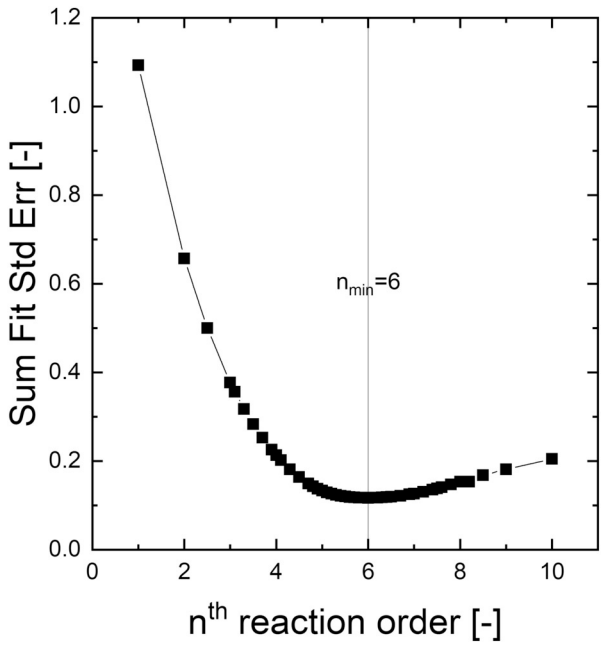

Fig. 4. Cumulative fitted standard errors of the modelled nth reaction order to the short thermal degradation kinetics of color active phycocyanin.

compared to highly purified cPC solutions applied in other literature. The applied *Spirulina* powder contained maltodextrin as carrier material from the spray drying process. Carbohydrates such as maltodextrin or trehalose are commonly applied carrier materials in spray drying *Spirulina* extracts for coloring food applications (Anzai, Fukuda, Kubo, & Sekiya, 2005; Heyde, Schiffelbein, & Christiansen, 2012; Iwao Kojima, Koji Odan, & Masahiro Nishikawa, 2013). They do not influence the photometric measurements and the detection of phycocyanin as shown by Yoshikawa and Belay (2008) from experiments with different *Spirulina* substances and phycocyanin spiked placebo matrices. Yet, detailed stability studies of the exact influence of carbohydrates on thermal phycocyanin stability in HTST processes should be considered in further studies. Literature (Antelo et al., 2008; Chaiklahan et al., 2012; Martelli et al., 2014) and industry practice (Anzai et al., 2005; Heyde et al., 2012; Iwao Kojima et al., 2013) suggest a stabilizing effect; however at 200–1000 times higher concentrations than the effective solubilized maltodextrin concentration of 0.056% (w/v) in this study.

Regarding the processing conditions, the presented measurements were performed in a batch process as well as with a continuous MMRS. As outlined previously, both processes rely on a high surface-to-volume ratio with 4444 m^{-1} in the batch process and 2610 m^{-1} in continuous operation, respectively, enabling fast heat transfers. This allowed controlled isothermal short holding times and fast heating and cooling. Differences to Chaiklahan et al. (2012) could originate in the distinctly larger sample volume of 30 ml as well as the not further specified temperature gradient box. Presumably, longer heating times could only be realized and inhomogeneities in temperature could have been an issue impacting the thermal treatments.

3.2. Modelling empirical decay reaction order

For thermal degradation of phycocyanin, the literature assumed first order linear $\log_{10}$ kinetics (Antelo et al., 2008; Chaiklahan et al., 2012; Patel et al., 2004). Kinetics that comply with a first order linear $\log_{10}$ behavior are characterized by a linear decay in a semi-logarithmic plot of decay variable over reaction time. This was not observed for the degradation of phycocyanin color activity in this study. The linear fits showed low coefficients of determination for batch processing at processing temperatures of 70, 75, and 80 °C with R^2 = 0.58, 0.48, and 0.41 and root mean square error (RMSE) = 0.150, 0.175, and 0.186, respectively; similarly for the linear fits of continuous processing R^2 = 0.67, 0.50 and 0.47 and RMSE = 0.159, 0.190, and 0.183, respectively.

Table 1
Fitting parameters k – reaction rate constant – and RMSE – Root Mean Square Error – of fits with reaction order n = 6 to relative color active phycocyanin concentration for batch processing in capillaries and for continuous processing in the modular micro reaction system (MMRS).

Parameter		k(T)	RMSE
Capillaries	70 °C	1.241	0.045
	75 °C	2.043	0.014
	80 °C	2.788	0.012
MMRS	70 °C	0.952	0.017
	75 °C	1.494	0.012
	80 °C	1.991	0.013

For T ≥ 70 °C, the decay in phycocyanin color activity is better described by a biphasic degradation behavior. It decreased within 30 s of processing time to 30% of the initial blue color intensity and tailed with increasing treatment time. As the observed kinetics diverge profoundly from a linear first order $\log_{10}$ reduction, the degradation data were modelled in an incremental approach to a non-linear decay with a varying reaction order from 1 to 10. The minimal cumulative standard fitted error was found for reaction order n = 6 (see Fig. 4), which resulted in the following Eq. (5) based on Eq. (3),

$$\frac{C_t}{C_0} = [1 + 5 \cdot k \cdot t]^{\frac{1}{-5}} \quad with\ n = 6 \tag{5}$$

Corresponding fitting curves are displayed in Fig. 3 with the logarithmic color active phycocyanin concentrations. The RMSE as a coefficient of determination and the fitted reaction rate constants are listed in Table 1. The RMSE, which is below 0.017 for all except one processing condition, stresses the goodness of fit with a reaction of the order n = 6. However, this high reaction order does not explain the color degradation on a mechanistic level. It is a purely empirical reaction order that describes the decay in color activity better than other

tested reaction orders. A high reaction order indicates that parallel reactions, consecutive reactions, or intermediate products are formed influencing the course of the investigated reaction so that reaction order n is better referred to as kinetic or apparent reaction order (Kessler, 2002).

In the case at hand, the degradation of phycocyanin's color activity upon thermal processing is likely an overall consequence of multiple contributing reactions. Spectral absorption of cPC and aPC is dominated by the conformation of the phycocyanobilin chromophores and how these chromophores interact in oligomers of cPC and aPC monomers due to spatial proximity (Berns & MacColl, 1989; Contreras-Martel et al., 2007). When the phycocyanobilin is bound to apoproteins of either cPC or aPC, the chromophore is in an extended conformation (Schirmer, Bode, & Huber, 1987). In this state the absorption spectrum depicts a characteristic main peak at 620 nm or 650 nm, respectively. If the protein structure denatures, the phycocyanobilin, a linear tetrapyrrole chromophore, relaxes and assumes a more stable cyclic conformation, which is characterized by a main absorption peak at 360 nm (Berns & MacColl, 1989). This shift in conformation can be explained by quantum chemical calculations (Scheer & Kufer, 1977).

Moreover, in aqueous low-ionic-strength conditions, aPC assembles in trimers, whereas cPC forms hexamers, trimers, or other oligomers under these conditions (MacColl, 1998; MacColl et al., 1981; MacColl, Berns, Traeger, & Csatorday, 1980). When disintegrated into monomers, absorption at 620 nm is slightly reduced in the case of cPC, whereas in the case of monomeric aPC the absorption spectra resembles the one of monomeric cPC, resulting in a shift of the main absorption peak from 650 nm to 615 nm (MacColl et al., 1980).

Described losses in proteins' quaternary structure could help to explain the observed characteristic alterations in absorption spectra with increasing processing time (see Fig. 2). Yet, absorbance at 360 nm would be unaffected by changes in the proteins quaternary structure alone. There must have also been changes in the tertiary structure of the apoproteins that led to a change of the phycocyanobilin from its extended state into its cyclic form with an absorption maximum at 360 nm (Scheer & Kufer, 1977). Hence, it is speculated that the observed degradation in color activity is a combined effect of the disintegration of cPC and aPC into monomers and a denaturation of monomers yielding phycocyanobilins in cyclic conformation. For further insights on a mechanistic level, studies must focus on purified phycocyanin fractions to omit side reactions and to allow for conclusions that are more specific.

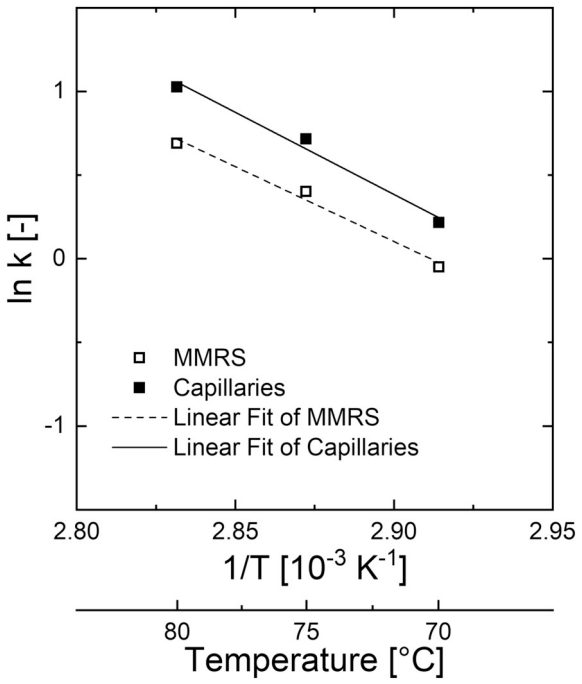

Fig. 5. Logarithmic reaction rate constant k plotted against the inverse of the absolute temperature for capillaries (batch processing) and modular micro reaction system (MMRS) (continuous processing). Temperature in Celsius as orientation. Reaction rate constant k was fitted to the thermal degradation data using the best fitting reaction order n = 6. The linear fits are characterized by $R^2 = 0.98$ and 0.99 for batch and continuous processing, respectively. The linear fits' slope equals the energy of activation E_a divided by the absolute gas constant R; $E_a = 81.6 \pm 10.3\,kJ\,mol^{-1}$ for batch processing and $E_a = 74.4 \pm 8.9\,kJ\,mol^{-1}$ for continuous processing.

Multiple reactions contributing to the degradation in color activity of phycocyanin solution would support the identified high empirical reaction order of n = 6. With the modelled apparent reaction order, the reaction rate constant k(T) could be derived for the different processing temperatures. By plotting the logarithmized reaction rate constant against the inverse of the absolute temperature (Fig. 5), the energies of activation (E_a) could be calculated from the Arrhenius equation (Eq. (4)). Thereby, an E_a of $74.4 \pm 8.9\,kJ\,mol^{-1}$ was derived for the continuous measurements and E_a of $81.6 \pm 10.3\,kJ\,mol^{-1}$ for the batch processing experiments. As the E_a are not significantly different from each other ($p = 0.41$), transfer could be demonstrated from batch processing in capillaries to continuous processing in the MMRS. This allows upscaling of the process through numbering-up approaches as parallelization of numerous micro-heating devices. Moreover, the provided knowledge of temperature-time interactions under isothermal conditions could support further upscaling initiatives by combining this with the respective heat and mass transfers, and flow conditions. Minor discrepancies in E_a could originate from heat transfer differences between the two systems. The continuous system implies conductive and convective heat transfer, whereas the batch heating in capillaries is dominated by conductive heat transfer. Residence times and thus processing times were similar in both systems due to high surface-to-volume ratios but also because of narrow residence time distributions reported with low viscous liquids for the MMRS system and its utilized modules (Georget et al., 2013). However, the experimental determination of the reaction order, which is the basis for calculation of the reaction rate constant k, relies on the spectrophotometric analytics of the processed phycocyanin solution. Color is the most important techno-functional property of phycocyanin powders for applications in the food industry, but its intensity degradation is an indirect description of the underlying chemical reactions as previously discussed. Improvements in determining the experimentally derived order of reaction require insights on a structural level and an understanding of the mechanistic processes.

4. Conclusion

An *Arthrospira platensis* extract with a high content in cPC and aPC was processed in thermal short time trials using a batch and a continuous heating system with very high surface-to-volume ratios; $4444\,m^{-1}$ and $2610\,m^{-1}$ for batch and continuous operation, respectively. Controlled thermal processing with treatment times up to 60 s showed a biphasic degradation of phycocyanin's color activity for treatment temperatures of $T \geq 70\,°C$. Revealed kinetics were faster than most kinetics described in the literature on thermal degradation of purified cPC and they were best described with a reaction order of n = 6. Supported with the high empirical reaction order, it is proposed that the degradation in color activity was due to a combination of reactions. Purified fractions of the phycocyanins need to be investigated to elucidate the structural and mechanistic changes of aPC and cPC in HTST processing. This study focused on an impure phycocyanin formulation that is applied in the food industry and could demonstrate the transfer from batch to continuous processing on a micro-processing scale. Consequently, the insights gained may help to improve multi category applications of phycocyanin, which is the only natural blue food coloring on the market to date.

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Conflict of interest statement

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