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Analytical Methods

Determination of the degradation kinetics of anthocyanins in a model juice system using isothermal and non-isothermal methods

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ABSTRACT

The effect of temperature on the degradation of blackcurrant anthocyanins in a model juice system was determined over a temperature range of 4–140 °C. The thermal degradation of anthocyanins followed pseudo first-order kinetics. From 4–100 °C an isothermal method was used to determine the kinetic parameters. In order to mimic the temperature profile in retort systems, a non-isothermal method was applied to determine the kinetic parameters in the model juice over the temperature range 110–140 °C. The results from both isothermal and non-isothermal methods fit well together, indicating that the non-isothermal procedure is a reliable mathematical method to determine the kinetics of anthocyanin degradation. The reaction rate constant (k) increased from 0.16 (±0.01) × 10^{-3} to 9.954 (±0.004) h^{-1} at 4 and 140 °C, respectively. The temperature dependence of the rate of anthocyanin degradation was modelled by an extension of the Arrhenius equation, which showed a linear increase in the activation energy with temperature.

1. Introduction

Anthocyanins are polyphenolic pigments, responsible for the red, blue and purple colours of many fruits. They are reported to have antioxidant properties and thus many health benefits (Kong, Chia, Goh, Chia, & Brouillard, 2003). Blackcurrants are an excellent source of anthocyanins and are of interest because they offer potential as an ingredient in functional beverages. Heat processing (e.g., pasteurisation, sterilisation) is an extremely common and effective method of preserving such beverages but may result in quality loss (Lund, 1982). Elevated temperatures can affect anthocyanin stability and cause monomeric anthocyanins to polymerise, resulting in browning (Markakis, 1982; Somers, 1966), which is undesirable in products such as fruit juices, where consumers perceive it as an indication of inferior quality.

There has been a lot of research on anthocyanin degradation based on isothermal methods at temperatures up to 100 °C (Cemeroglu, Velioglu, & Isik, 1994; Kirca, Ozkan, & Cemeroglu, 2007; Markakis, Livingstone, & Fellers, 1957; Reyes & Cisneros-Zevallos, 2007; Wang & Xu, 2007), mainly using first-order kinetics, and modelling the temperature dependence using the Arrhenius equation. However, most industrial thermal sterilisation processes (e.g., retorting, ultra high temperature (UHT)) involve temperatures above 100 °C, yet no kinetic data has been published at these temperatures. Ideally, degradation kinetics of these compounds needs to be established up to 140 °C.

At lower temperatures isothermal methods can be used to estimate degradation kinetics as the ramp-up and ramp-down times are negligible compared to the length of the holding phase. However, above 100 °C isothermal methods cannot be used to estimate degradation kinetics as the duration of the heat-up and cool-down phases are too large to be ignored. Therefore, to estimate the kinetic parameters at these higher temperatures, non-isothermal methods need to be used. Recently, Dolan (2003) put forward a convenient method for calculating the degradation kinetic parameters for non-isothermal food processes, based on the Arrhenius model. The method involves combining the independent variables time (t) and temperature (T) into one variable, the thermal history (β). The initial (C_0) and final (C) concentrations, and the thermal history can then be used to determine the activation energy (E_a) and the reaction rate constant (k), by minimising the sum of squares error (SSE).

In this study, anthocyanin concentrations were assessed using the pH differential method developed by Giusti and Wrolsted (2001). This method measures the absorbance at two pH values (pH 1 and 4.5) and is based on the change in anthocyanin structure as a function of pH. The objective of this experiment was to assess the heat stability of blackcurrant anthocyanins in a functional drink-type product, by using both isothermal and non-isothermal methods to determine the kinetic parameters.

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2. Materials and methods

2.1. Materials

Sucrose and citric acid were purchased from Merck (Darmstadt, Germany). Food grade potassium sorbate was obtained from Hoechst GmbH (Frankfurt am Main, Germany). Analytical grade potassium chloride (Merck, Darmstadt, Germany) and sodium acetate trihydrate (Fisher, Loughborough, UK) were used to make buffer solutions. Mineral water was purchased from Ballygowan (Limerick, Ireland) and individually quick frozen (IQF) blackcurrants (*Ribes nigrum* L.) were purchased from Boylans fruit (Kildare, Ireland) and stored in a cold room at −18 °C until required for product manufacture.

2.2. Blackcurrant juice preparation

The model juice was based on the formulation of Dyrby, Westergaard, and Stapelfeldt (2001). The quantities and ingredients used are presented in Table 1. The blackcurrants were blanched in boiling water for 2 min, to increase anthocyanin extraction (Lee, Durst, & Wrolstad, 2002), and then immediately cooled in an ice-bath to stop thermal degradation. All ingredients were blended at full speed in a food processor (Model No. KM800, Kenwood, Havant, UK) for 2 min. The juice was filtered through a 300 μm sieve and then centrifuged at 1500 RCF for 15 min at 20 °C, to reduce its turbidity. The juice had a pH of 3.4 and 11° Brix.

2.3. Heat treatment

Aliquots of blackcurrant juice (10 ml) were put into capped Pyrex tubes (150 × 16 mm) with a wall thickness of 2 mm. For the isothermal experiments the juice was put in a constant temperature room at 4 °C and 21 °C or heat treated in a forced air oven (Model No. FD 115/E2, Binder GmbH, Neckarsulm, Germany) at 40, 60, 80 and 100 °C. In order to ensure a short come-up time at high temperatures (60–100 °C), the samples were heated in a water-bath until the desired temperature was within 1 °C and then transferred to the oven. This ensured that the come-up times were relatively short compared to the overall durations of the experiments (less than 5% in all cases). At each temperature, samples (n = 8–12 independent replicates per temperature) were tested for anthocyanin content at regular time intervals. For the non-isothermal experiments, each sample was heated in an autoclave (Rodwell Scientific Instruments, Basildon, UK) at a range of temperatures from 110 °C to 140 °C. The temperatures of the juice samples were recorded at 1 min intervals during cooking with a temperature logger (Model No. MMS3000-GP4, ISE, Inc., Cleveland, OH) and a fast response flexible Type *K* thermocouple with a diameter of 0.64 mm, teflon coating and a welded tip (Industrial Temperature Sensors, Naas, Ireland), which was placed into one sample test tube to represent each lot of tubes. The temperature profiles of the model juice heat-treated in the autoclave are shown in Figs. 1 and 2. Samples were heated at 110 °C for 1, 10, 20 and 30 min and then tested (Fig. 1).

Table 1
Formulation of the model blackcurrant juice used in the present study

Ingredient	Quantity (g)
Blackcurrants	250
Sucrose	86
Citric acid	1.52
Potassium sorbate (preservative)	0.18
Mineral water	662.30
Total	1000

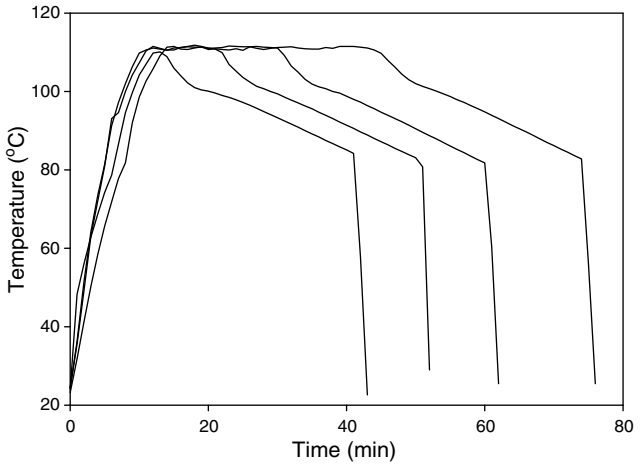

Fig. 1. Temperature profiles from samples heated to 110 °C and held at this temperature for 1, 10, 20 and 30 min.

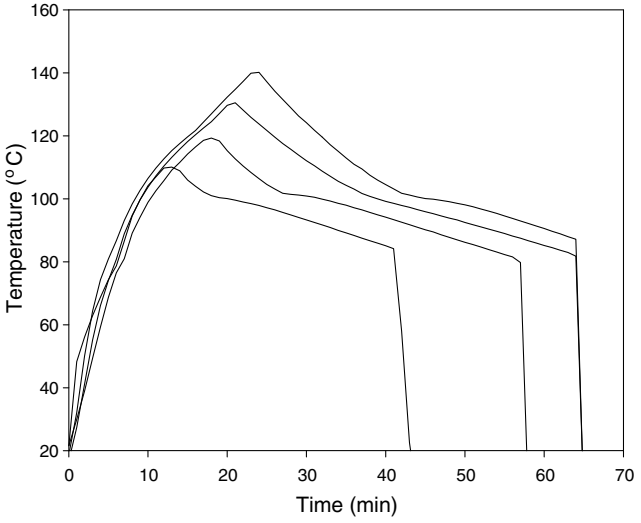

Fig. 2. Temperature history for samples heated to 110, 120, 130 and 140 °C and held at these temperatures for 1 min.

From these four temperature profiles, 11 independent replicates were generated by treating some samples twice (i.e., 0, 1, 10, 20, 30, 1 + 10, 1 + 20, 1 + 30, 10 + 20, 10 + 30, 20 + 30 min). These time–temperature combinations were chosen to mirror those used during retorting in the canning industry. To represent time–temperature combinations used in UHT processing, another sample set was heated at 110–140 °C for 1 min and then tested (Fig. 2). Figs. 1 and 2 clearly show the temperature profiles achieved by the model juice in the autoclave, which allowed for a fast heating rate (~8 °C min^{-1}) to the desired temperature, followed by the required holding time. The temperature of the sample then dropped sharply to 100 °C, due to the fast release of steam from the autoclave chamber and the return to atmospheric pressures and then more slowly to 80 °C, at which point the autoclave was opened and the sample was put in an ice-bath to stop further thermal degradation.

2.4. Determination of anthocyanins

Total monomeric anthocyanins were calculated by the pH-differential method, described by Giusti and Wrolsted (2001). The absorbance of the juice was measured at pH 1 and 4.5 using a

UV–visible spectrophotometer (Helios, Unicam, Cambridge, UK) at the wavelength of maximum absorbance (520 nm) and also at 700 nm to correct for haze. Calculations were based on cyanidin-3-glucoside with molecular weight of 449.2 g mol^{-1} and molar absorbance of 29,600 cm^{-1} mol^{-1} l. All measurements were repeated in triplicate.

2.5. Degradation kinetics modelling

2.5.1. Isothermal model

The two-step regression model was used to determine the kinetic parameters for the samples treated isothermally. Pseudo first-order kinetic rate constants (k) at each temperature were calculated by plotting ln (C) against time, where C is the concentration of anthocyanins, (Eq. (1)):

$$C = C_0 \exp(-kt) \quad (1)$$

The half-lives of the anthocyanins ($t_{1/2}$) were calculated using Eq. (2) below:

$$t_{1/2} = \frac{\ln(2)}{k} \quad (2)$$

The Arrhenius activation energy (E_a) was calculated by plotting ln (k) against $1/T$ (absolute temperature in Kelvin).

2.5.2. Non-isothermal model

The non-isothermal model was based on the 1-step method put forward by Dolan (2003). The independent variables time (t) and model juice temperature (T) were combined into one variable, the thermal history (β), according to Eq. (3):

$$\beta = \int_0^t \exp\left[\frac{-E_a}{R}\left(\frac{1}{T_{(t)}} - \frac{1}{T_r}\right)\right] \quad (3)$$

where T_r is the arbitrary reference temperature and $T_{(t)}$ is the temperature (T) at time (t).

The measured C/C_0 values could then be plotted as a function of β. The reaction rate constant (k_r) at T_r could then be determined and a set of C/C_0 values calculated according to Eq. (4):

$$\left(\frac{C}{C_0}\right)_{Calc} = \exp(-\beta \times k_r) \quad (4)$$

The kinetic parameters (k_T and E_a) were calculated by minimising the sum of squares error (SSE) between the experimental and calculated values for C/C_0 by using Solver in Microsoft® Excel.

3. Results and discussion

3.1. Kinetics of anthocyanin thermal degradation

3.1.1. Isothermal method

Fig. 3 shows the relationship between the concentration of monomeric anthocyanins and time, over the temperature range of 4–100 °C. From the graph it is clear that anthocyanin concentration decreased with time at all temperatures and that anthocyanins are more rapidly degraded at higher temperatures. When the logarithm of the concentration of monomeric anthocyanins was plotted against time, a straight line resulted (results not shown), indicating pseudo first-order reaction kinetics for anthocyanin degradation. This is in agreement with previous reports (Cemeroglu et al., 1994; Kirca & Cemeroglu, 2003; Markakis et al., 1957; Wang & Xu, 2007).

The first-order reaction rate constants (k) and half-lives ($t_{1/2}$) of the model juice are indicated in Table 2. In all cases the r^2 values were higher than 0.97, indicating a good data fit to the first-order kinetic model. The k and $t_{1/2}$ values further confirmed the influence of temperature. The k values increased with temperature from 0.16 (±0.01) × 10^{-3} at 4 °C to 310 (±5) × 10^{-3} h^{-1} at 100 °C. The corresponding $t_{1/2}$ values decreased from 180 ± 11 days to 2.18 ± 0.04 h, as the temperature increased from 4 to 100 °C. Other authors have also reported that k values increase with increasing temperature and $t_{1/2}$ values decrease (Cemeroglu et al., 1994; Kirca et al., 2007; Kirca & Cemeroglu, 2003; Wang & Xu, 2007). Cemeroglu et al. (1994) found that for sour cherry juice the $t_{1/2}$ values were 54.34 and 8.1 h at 60, and 80 °C, which indicates that sour cherry anthocyanins are more heat stable than those in the blackcurrant juice. Kirca and Cemeroglu (2003) reported the $t_{1/2}$ values for blood orange juice to be 3.6 h at 80 °C. Wang and Xu (2007) found that the $t_{1/2}$ values of blackberry juice were 16.7 and 4.7 h at 60 and 80 °C. Therefore anthocyanins in blackcurrant seem more heat stable than those in blackberry or blood orange juice. The activation energy (E_a) of the blackcurrant juice was 73 ± 2 kJ/mol (calculated over the range 21–100 °C) and is very close to the E_a values reported by Cemeroglu et al. (1994) for sour cherry juice of 15° Brix (68.52 kJ/mol) and to the values reported by Kirca and Cemeroglu (2003) for blood orange juice of 11.2° Brix (73.60 kJ/mol). The E_a value reported by Wang and Xu (2007) for blackberry juice was slightly lower (58.95 kJ/mol).

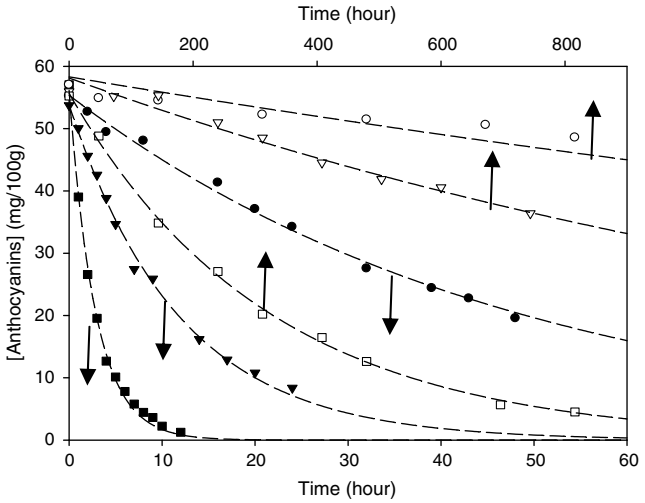

Fig. 3. Evolution of the concentration of monomeric anthocyanins with time at the temperatures 4 (○), 21 (▽), 40 (□), 60 (●), 80 (▼) and 100 (■) °C (Note: broken lines represent the behaviour predicted by the pseudo first-order kinetic model).

Table 2
Effect of temperature on the k and $t_{1/2}$ values of anthocyanin degradation in the model blackcurrant juice

Temperature (°C)	k 10^3 (h^{-1})	$t_{1/2}$ (h)	r^2
4	0.16 ± 0.01	180 ± 11[a]	0.9740
21	0.61 ± 0.03	49 ± 2[a]	0.9947
40	3.1 ± 0.1	9.4 ± 0.3[a]	0.9968
60	21 ± 3	32 ± 5	0.9955
80	78 ± 2	9 ± 0.2	0.9947
100	310 ± 5	2.18 ± 0.04	0.9954

[a] Days.

3.1.2. Non-isothermal method (constant holding temperature and varied holding time)

The kinetic parameters of the model juice at 110 °C were calculated by minimising the sum of squares error (SSE) between the 11 experimental and calculated values for C/C_0 (Fig. 4). The values for k_T at 110 °C and E_a were determined to be 1.02 ± 0.02 h^{-1} and 81.51 ± 0.03 kJ/mol, respectively. The r^2 value for k_T and E_a was found to be 0.98, indicating a good fit between experimental and

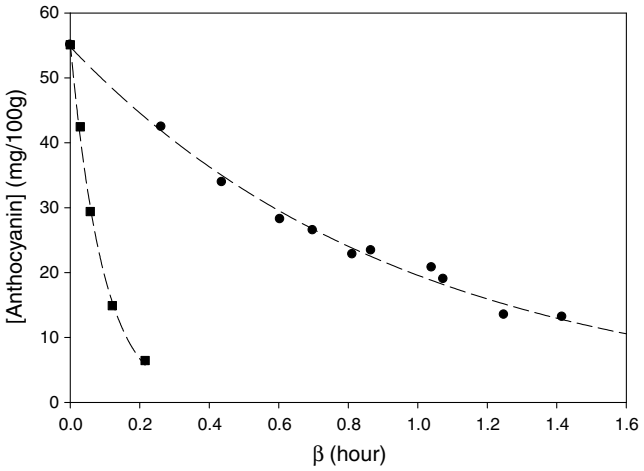

Fig. 4. Concentration of anthocyanins plotted against the thermal history (β) at 110 °C (●) and 140 °C (■) (Note: broken lines represent the behaviour predicted by the pseudo first-order kinetic model).

calculated values. Fig. 4 shows anthocyanin concentration plotted against the thermal history (β) at 110 °C and 140 °C. As expected, anthocyanins degrade more rapidly at 110 °C and 140 °C than at the lower temperatures (4–100 °C).

3.1.3. Non-isothermal method (constant holding time and varied holding temperature)

The kinetic parameters of the model juice at a reference temperature (T_r) of 140 °C were also calculated using the samples heated at a constant holding time (1 min) with varying temperature (110, 120, 130 and 140 °C) as in Fig. 2. Again, E_a and k_T were calculated by minimising the SSE between the five experimental and calculated values for C/C_0. The resulting values were found to be 91.09 ± 0.03 kJ/mol and 9.954 ± 0.004 h^{-1}, respectively. The r^2 value was 0.99, which indicates a very good fit between experimental and calculated values for C/C_0, as can be seen in Fig. 4.

3.2. Comparison of isothermal and non-isothermal methods

The Arrhenius plot for anthocyanin degradation in the model juice over a temperature range of 4–140 °C is shown in Fig. 5. It illustrates the temperature dependence of monomeric anthocyanin degradation under both isothermal (4–100 °C) and non-isothermal (up to 110 °C and 140 °C) conditions. Overall both the isothermal and non-isothermal results fit well together on the graph (r^2 = 0.99) indicating that the non-isothermal method proposed by Dolan (2003) is a reliable mathematical method to determine the kinetics of anthocyanin degradation. However, it was noted that the k values at the extremes (i.e., 4 °C and 140 °C) were both slightly above the regression line compared to the other results. The overall E_a was calculated to be 81 ± 3 kJ/mol, which is not significantly different ($p \geqslant 0.05$) to the E_a at a temperature equivalent of 110 °C (81.51 ± 0.03 kJ/mol). The E_a value determined from the experiments conducted at 140 °C was 91.09 ± 0.03 kJ/mol. Thus, the E_a value seems to increase with increasing temperature. This increase has already been reported by other workers in the literature. Cemeroglu et al. (1994) performed separate analyses on the effect of storage temperature (5–37 °C) and heating temperatures (50–80 °C) on the degradation kinetics of sour cherry anthocyanins. At a concentration of 45° Brix the E_a at storage temperatures was 65.22 kJ/mol and it increased to 75.89 kJ/mol at heating temperatures. Similarly, Kirca and Cemeroglu (2003) studied degradation kinetics in blood orange juice at a concentration of 45° Brix and found the E_a at storage temperatures (5–37 °C) was 73.2 kJ/mol and the E_a at heating temperatures (50–80 °C) was 84.5 kJ/mol. This apparent temperature dependence of the activation energy is not unusual for complex solvolytic reactions (Blandamer, Burgess, Robertson, & Scott, 1982; Euranto & Kanerva, 1983) and is consistent with an anthocyanin degradation mechanism where several chemical species exist in equilibrium (Brouillard, 1982). We therefore recalculated the activation energy at each temperature according to Eq. (5) which corresponds to the Robinson model in the Blandamer review (Blandamer et al., 1982):

$$E_a = mT + b \tag{5}$$

The resulting E_a values over the entire temperature range studied are plotted in Fig. 6, together with the values for blood oranges and grapes recalculated from the literature (Kirca & Cemeroglu, 2003; Reyes & Cisneros-Zevallos, 2007). This very good match between the values of the present study and the literature, together with the fact that all activation energies seem to follow the same temperature dependence tend to indicate that this model adequately describes the overall kinetic parameters of anthocyanin degradation over a wide range of temperatures and also for various fruits. Because of the variety in chemistry of the anthocyanins present in the blackcurrant juice studied, no attempt can be made here to interpret further these kinetic parameters, in order to elucidate the possible mechanisms of anthocyanin degradation.

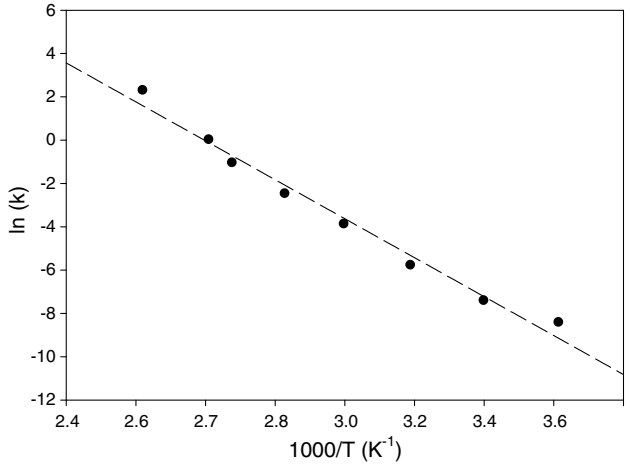

Fig. 5. Arrhenius plot for anthocyanin degradation in the model juice over a temperature range of 4–140 °C.

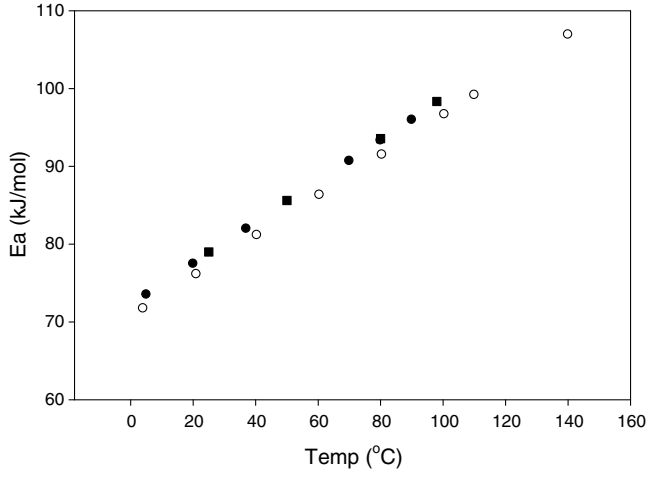

Fig. 6. The evolution of E_a over the temperature range 4–140 °C for blackcurrant anthocyanins (○) (present study), grape anthocyanins (■) (Reyes & Cisneros-Zevallos, 2007) and blood orange juice (●) (Kirca & Cemeroglu, 2003).

Nevertheless, to determine anthocyanin degradation at typical food industry sterilisation temperatures (>100 °C) it is important to use non-isothermal methods at these high temperatures because the degradation kinetics cannot be extrapolated without significantly underestimating the anthocyanin degradation.

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