

Kinetic modelling of non-enzymatic browning of apple juice concentrates differing in water activity under isothermal and dynamic heating conditions

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Abstract

The kinetics of non-enzymatic browning in apple juice concentrates were investigated. The effect of a_w (in the range of 0.74–0.99) and/or reactant concentration on brown pigment formation was monitored under isothermal heat treatment at four temperatures (60, 70, 80 and 90 °C) in apple juice solutions having either the same or different concentrations of reactant solutes. The extent of the Maillard reaction was evaluated by spectrophotometric measurements at 420 nm (A_{420}). The absorbance–time curves were fitted to five different kinetic models (zero and first order, weibull, logistic and the parabolic model) and estimates of browning rate constants and other model parameters were obtained. Regression analysis revealed that the logistic model was the most appropriate for describing browning in apple juice. The initial reactant concentration, but not water activity, had a significant effect on the colour change of apple juice. The processing temperature also had a strong impact on browning kinetics. Secondary models, expressing the dependence of the best fitted primary model parameters on temperature and a_w , were further developed and validated by comparing the predicted model parameters with the values observed in independent isothermal experiments. Finally, the derived model was further evaluated against the observed browning responses of apple juice under dynamic heating conditions, underlining the applicability of the developed model as a practical prediction tool for the study of non-enzymatic browning.

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1. Introduction

Thermal processing is commonly practised to extend the shelf life of fruit products. However, heating processes can affect the quality of product, leading to consumer dissatisfaction (Rattanathanalerk, Chiewchan, & Srichumpoung, 2005). Such problems are often attributed to non-enzymatic browning reactions that involve caramelization, ascorbic acid degradation and formation of Maillard reaction prod-

ucts. The Maillard reaction taking place between α -amino groups and reducing sugars is the most important cause of browning in apple juice (Toribio & Lozano, 1984). Maillard browning may be desirable during processing of some food products, as in the manufacture of coffee, tea, beer and bread baking, since it improves colour, aroma, and flavour (Carabasa-Giribet & Ibarz-Ribas, 2000; Eskin, 1990; Martins, Jongen, & van Boekel, 2001). On the other hand, it may be undesirable in concentrated, intermediate moisture and dried foods, since maximum reaction rate occurs at water activities of 0.6–0.7 (Eskin, 1990). In addition, it causes loss of nutrients (e.g lysine), formation of undesirable compounds, e.g. furfural and 5-hydroxymethylfurfural

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(HMF), and finally brown pigment formation (Buedo, Elustondo, & Urbicain, 2001; Labuza & Baiser, 1992; Martins et al., 2001).

Several methods have been suggested for quantifying the development of browning components, including both colour measurements and chemical analysis. Visual colour change of fruit products has often been described by recording the CIE-Lab colour indices (Burdurlu & Karadeniz, 2003; Garza, Ibarz, Pagan, & Giner, 1999; Ibarz, Pagan, & Garza, 1999; Ibarz, Pagan, & Garza, 2000). Spectrophotometric measurements, and in particular absorbance at 420 nm, is the most common method for brown pigment detection (Labuza & Baiser, 1992), while the formation of 5-hydroxymethylfurfural has been widely used as an indicator to monitor intermediate stage products (Bozkurt, Gogus, & Eren, 1999; Burdurlu & Karadeniz, 2003; Cohen, Birk, Mannheim, & Saguy, 1998; Garza et al., 1999; Ibarz et al., 1999; Ibarz et al., 2000; Rattanathanalerk et al., 2005). Finally, fluorescence measurements have also been reported in the literature as a quality deterioration index of apple juice during thermal processing (Cohen et al., 1998).

The complexity of Maillard reaction, consisting of many parallel and consecutive reactions with various components, reveals a great difficulty in studying the phenomenon by means of a simple analytical chemical method that would adequately describe the whole process. The most common method for characterizing browning is the measurement of colour development as a function of time and its expression in terms of kinetics of the reaction, which is described by the reaction rate constant. Many researchers have reported the appearance of brown pigments as following either zero order or first order kinetics (Cohen, Birk, Mannheim, & Saguy, 1994; Stamp & Labuza, 1983; Toribio & Lozano, 1986). An important aspect is also to know the effect of temperature on browning kinetics and how the chemical environment of a food system, including water activity, pH and chemical composition, affects the kinetics of the reaction (Carabasa-Giribet & Ibarz-Ribas, 2000). Browning rate is strongly influenced by environmental factors, such as temperature and water activity. With respect to moisture content, the accepted scenario is that the rate of browning increases from the dry state, starting at a critical a_w of 0.2–0.3 for most foods, to a maximum at water activity of 0.5–0.8 and then decreases at higher water activities as a result of dilution of the reactant species (Labuza & Baiser, 1992).

Although many researchers have claimed that browning rate is not simply related to water activity, but rather to the amount of water and its state (degree of binding) in a particular system, no studies have dealt with the individual effect of a_w in liquid systems under a fixed concentration of reactant solutes. In this study, kinetic modelling was used to investigate non-enzymatic browning of heated apple juice concentrates in preparations having either the same or different initial reactant concentrations in the same range of water activity variation. The main objectives were:

(a) to evaluate the effect of reactant solutes and a_w on the kinetics of browning, (b) to develop a kinetic model able to describe the extent of browning as a function of time, temperature and a_w under isothermal heating conditions and (c) to determine whether the model developed using data derived from steady state heating conditions could be applied to predict actual browning of apple juice under non-isothermal heating with fluctuating temperature-time processing protocols.

2. Materials and methods

2.1. Sample preparation

A commercial apple juice concentrate (a_w 0.740, 70 °Bx) was offered by a Greek juice processing firm and kept in the freezer (−20 °C) for the entire experimental time period. This product was the concentrated parent solution for all sample preparations used in this study. Sorbitol was purchased from Sigma–Aldrich Co. (Gillingham, Dorset).

The initial apple juice concentrate, warmed first to room temperature, was either diluted with distilled water or mixed with sorbitol solutions (the variation of sorbitol concentration led to variations in the water activity of the solutions produced) to obtain ten (10) solutions of apple juice in the same range of a_w levels (0.74, 0.82, 0.87, 0.93 and 0.99). These two groups of solutions differed in the initial concentration of reactant sugars. In the first group of juice solutions (prepared after dilution with distilled water), the reactant sugars content varied from 70 to 11.65 °Bx, following, respectively, the above mentioned water activity range. However, the second group of juice solutions (those where the concentrated juice was mixed with appropriate portions of sorbitol–water solutions, keeping the volume ratio of the mixed liquids constant) had the same concentration of reactant sugars, starting with an initial diluted juice system fixed at 11.65 °Bx. The use of sorbitol as a non-reacting humectant allowed evaluation of the a_w effect on brown colour development independently of reactant concentration.

Water activity measurements (a_w) of all samples at initial and final treatment times were made using a water activity meter (Aqualab, Model series 3TE, Decagon Devices Incorporation, Pullman, Washington) at 20 °C.

2.2. Isothermal heating and spectrophotometric measurements

The ten apple juice preparations, differing in a_w and/or concentration of reactant sugars, were placed in hermetically sealed glass test tubes and heated isothermally at 60, 70, 80 or 90 °C. At each sampling time, two replications at every temperature and a_w combination were conducted. At proper time intervals, the tubes were withdrawn, rapidly cooled on ice and stored in the freezer until the time of analysis. All concentrated apple juice samples were diluted to 11.65 °Bx (solid contents at the level of commercial

reconstituted juice) prior to analysis. The extent of brown pigment formation was evaluated spectrophotometrically on a UV–Vis spectrophotometer (SP-8001 UV–Vis, Meter-tech Inc.) by measuring the absorbance in 10 mm cells against water at 420 nm as a function of time. All samples were further diluted, when necessary, to obtain reliable absorbance readings in the range of 0–1.8 units, to satisfy the linearity requirement between absorbance readings and concentration of colorants, according to Beer's law. Thus, eighty (80) absorbance–time curves were derived for the description of non-enzymatic browning (NEB) kinetics of apple juice samples.

In addition, in an independent experiment, three apple juice samples with a_w levels of 0.74, 0.85 and 0.96, prepared by dilution of the concentrated apple juice with distilled water, were heated isothermally at 65, 75 or 85 °C. The kinetics of brown pigment formation, as absorbance measurements at 420 nm, were again followed. The data obtained from this independent experiment were used for the validation of the model.

It is important to mention that, although the long treatment times used in this study are rather excessive for actual food processing protocols, they are useful for getting a complete kinetic description of the browning reaction.

2.3. Model development

The data from 80 absorbance–time curves (four heating temperatures, five a_w levels, two groups of solutions and two replications for each of the above combinations) were fitted to five different primary kinetic models, using the TableCurve™ 2D software (v4, SPSS Inc.) according to the following equations: the pseudo – zero order model,

$$A = A_0 + kt \quad (1)$$

the pseudo – first order model,

$$A = A_0 \times \exp(kt) \quad (2)$$

the parabolic model (Buedo et al., 2001),

$$A = \left(\sqrt{A_0} + kt\right)^2 \quad (3)$$

the weibull model,

$$A = A_{\max} + (A_0 - A_{\max}) \exp[-(kt)^\beta] \quad (4)$$

and the logistic model,

$$A = A_0 + \frac{A_{\max} - A_0}{1 + \exp[-k(t - t_i)]} \quad (5)$$

where A is the absorbance measured at 420 nm at time t , A_0 is the initial absorbance ($t = 0$) at the same wavelength, k is the browning rate constant (h^{-1}), A_{\max} is the maximum absorbance, β is the shape constant in the weibull model (Eq. (4)) and t_i is the time (in h) when half of the maximum absorbance is reached in the logistic model (Eq. (5)).

The estimates of browning reaction rate constants (k) and other model parameters were then fitted to secondary

models. Four different models describing the individual and combined effects of temperature and a_w on k were used and comparatively evaluated.

One of the models was the following modified Arrhenius model:

$$\ln k = \ln k_{\text{ref}} + d(a_w - a_{w\text{ref}}) - \frac{E_\alpha}{R} \left(\frac{1}{T} - \frac{1}{T_{\text{ref}}} \right) \quad (6)$$

where T is the absolute temperature (K), E_α is the activation energy (kJ/mol), R is the universal gas constant (8.314 J/mol K), T_{ref} is the reference temperature (373 K or 100 °C), $a_{w\text{ref}}$ is the reference water activity equal to 1, k_{ref} is the rate constant value (h^{-1}) at reference temperature and reference a_w and d is a constant expressing the effect of water activity on k .

Another model, based on the general Belehradek equation for biological growth rate, and here expressing temperature and a_w dependence of the non-enzymatic browning rate constant, given by the following equation, was examined:

$$\sqrt{k} = b(T - T_{\min})\sqrt{a_{w\text{max}} - a_w} \quad (7)$$

where T is the temperature (°C), T_{\min} and $a_{w\text{max}}$ are estimated parameters expressing the theoretical minimum temperature and maximum a_w for browning to commence, while b represents a constant.

Finally, two quadratic response surface models fitted either estimated k values or the square root of k values, as expressed by the polynomial equations given below:

$$k = a_1 + (a_2 \times T) + (a_3 \times a_w) + (a_4 \times T \times a_w) + (a_5 \times T^2) + (a_6 \times a_w^2) \quad (8)$$

$$\sqrt{k} = a_1 + (a_2 \times T) + (a_3 \times a_w) + (a_4 \times T \times a_w) + (a_5 \times T^2) + (a_6 \times a_w^2) \quad (9)$$

where T is the heating temperature (°C), and a_1 through a_6 are estimated coefficients.

Secondary models were also fitted to other parameters derived from the primary models, such as maximum absorbance A_{\max} and t_i in the case of the logistic model. The modified Arrhenius model and the polynomial equations of A_{\max} , $\ln A_{\max}$, t_i , and $\ln t_i$ were again tested for expressing the individual and combined effects of temperature and a_w (Eqs. (6) and (8)) on these parameters.

TableCurve™ 3D software (v3, SPSS Inc.) was used for fitting of the secondary models (Eqs. (6)–(9)) and analysis of variance. The statistically significant parameters of the models were determined by stepwise regression analysis (F -value).

2.4. Model validation

The obtained secondary mathematical models describing the rate constant, k , as the most important parameter were validated by comparing predicted browning rate constants with values observed in independent experiments at

three different heating temperatures (65, 75, 85 °C) and a_w levels (0.74, 0.85, 0.96). Finally, the general model derived from the experimental data was validated against measured brown pigment formation under non-isothermal heating conditions using different dynamic temperature scenarios of periodic temperature changes from 65 to 85 °C. The fluctuating time–temperature protocols studied here were recorded using a Hobo U12 Stainless Temp Data Logger (Onset Computers Corporation, southern MA USA) and the accompanied Hoboware™ software (version2).

3. Results and discussion

3.1. Absorbance evolution under isothermal heating conditions

Absorbance at 420 nm has generally been used to determine non-enzymatic browning in fruit juices as a quick and easy method for monitoring the extent of the reaction (Bozkurt et al., 1999; Burdurlu & Karadeniz, 2003; Cohen et al., 1998; Garza et al., 1999; Ibarz et al., 1999; Johnson, Braddock, & Chen, 1995; Koca, Burdurlu, & Karadeniz, 2003; Manso, Oliveira, Oliveira, & Frias, 2001; Nagy, Lee, Rouseff, & Lin, 1990; Rattanathanalerk et al., 2005; Toribio & Lozano, 1984). Isothermal heating of all apple juice samples progressively resulted in juice darkening and formation of brown pigments as recorded by the absorbance evolution during the heating period. With respect to temperature dependence of NEB kinetics of apple juice concentrates, Fig. 1 reveals the strong impact of increasing the processing temperature on the rate of colour development. The temperature effect was similar for solutions having either the same (Fig. 1a) or different initial concentrations of reactant sugars (Fig. 1b), with the latter case being the more pronounced. On the other hand, regarding the effect

of a_w on NEB reaction kinetics, some interesting observations were made. Solutions with the same initial concentration of reactant sugars (Fig. 2a and b), gave similar browning kinetic plots, regardless of their differences in water activity; the effect of a_w for the group of solutions with incorporated sorbitol was not statistically significant ($P > 0.05$). In contrast, the juice concentrates diluted with water exhibited higher browning rate constants with decreasing water activity (Fig. 2c and d). This classic behaviour of brown pigment formation enhancement of concentrated systems has also been reported by other researchers (Baisier & Labuza, 1992). Toribio and Lozano (1984), investigating non-enzymatic browning of apple juice concentrates, reported advanced colour development with increasing solid content (from 65 to 70 and finally 75 °Bx) and/or decreasing water activity at all storage temperatures tested. The same authors in a later study (Toribio & Lozano, 1986) found that the browning reaction rate of clarified apple juice depended, among other factors on soluble solids, and critically on the total amino acid content. Comparable, higher rate constants for non-enzymatic browning in heated orange juice and orange juice serum with increasing solid concentration were reported by Johnson et al. (1995).

Water activity is frequently mentioned as an important factor affecting non-enzymatic browning reaction rates. The question is whether or not this is a specific effect of water activity, or more an effect of concentration and diffusion. In general, the rate of browning increases from the dry state to a maximum at a water activity of 0.5–0.8 and then decreases at higher water activities (Labuza & Baisier, 1992). Solute mobility is limited below the monolayer due to the higher binding energy and the glassy state that forms (Levine & Slade, 1989). When the water activity is increased, the solutes become more mobile, they can

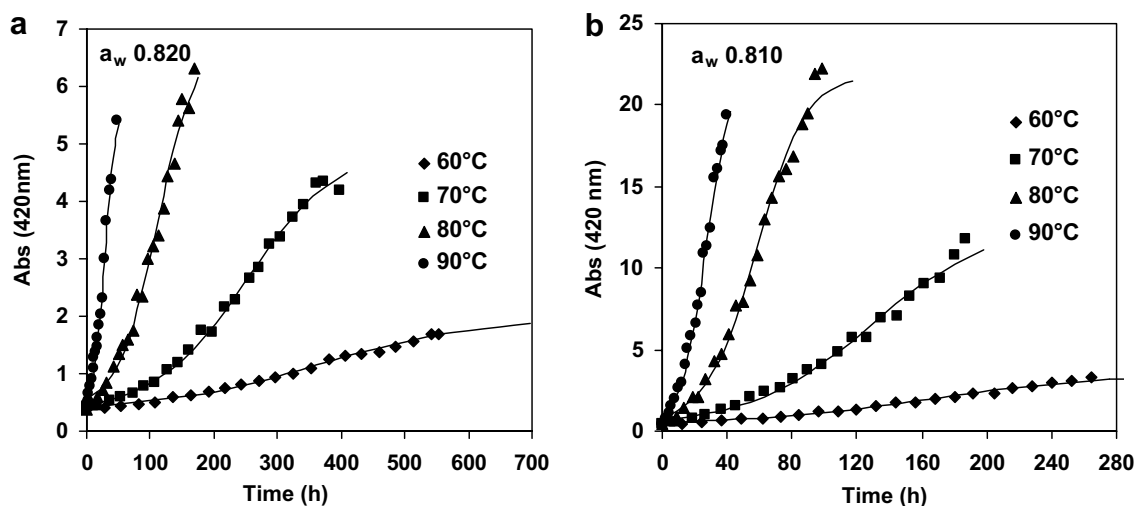


Fig. 1. Absorbance–time plots of browning kinetics for apple juice concentrates of similar a_w but varying in reactant sugar concentration at different heating temperatures: (a) diluted juice concentrate adjusted to the denoted a_w level with the addition of sorbitol; (b) juice concentrate diluted with distilled water to the denoted a_w .

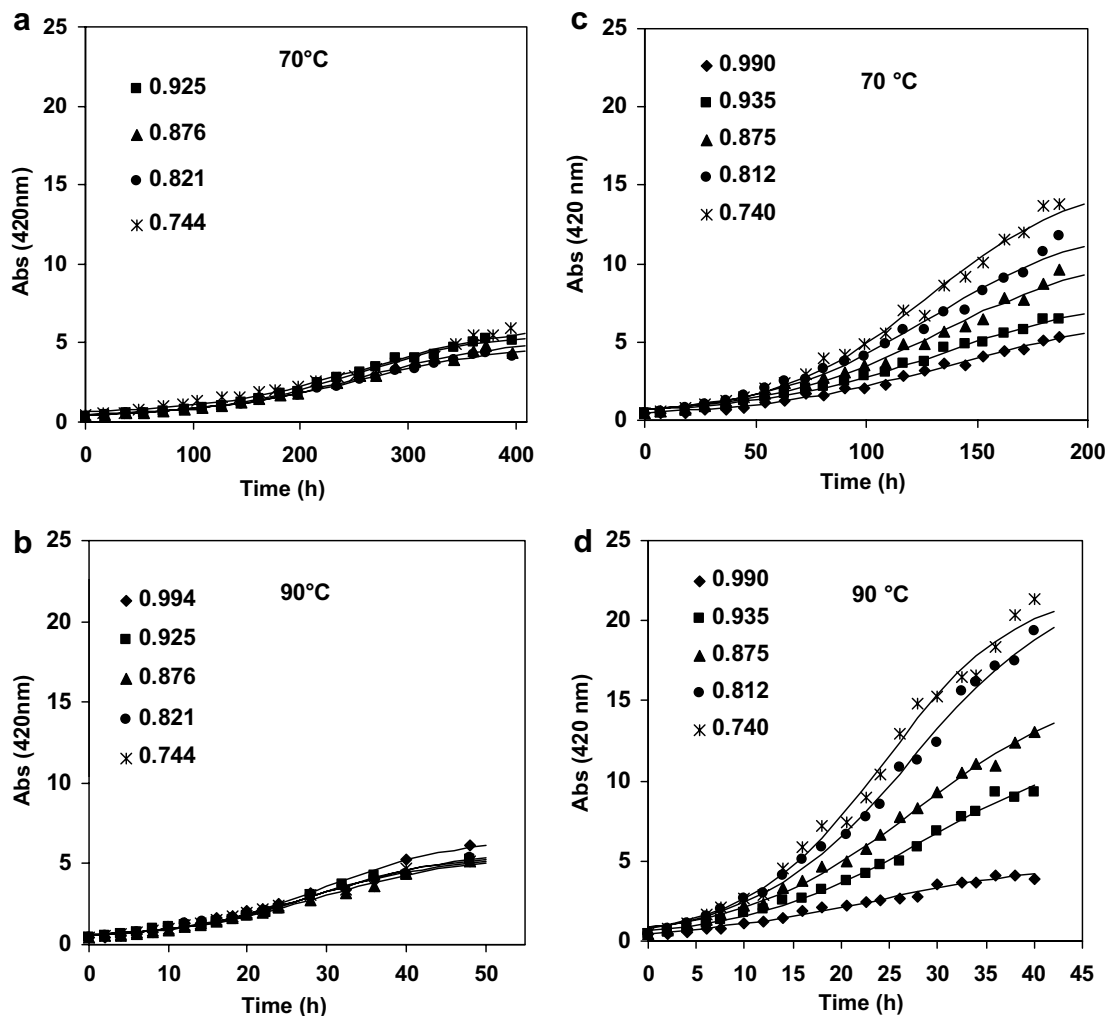


Fig. 2. Absorbance–time plots of browning kinetics for apple juice concentrates differing in a_w and having the same (a, b) or different (c, d) initial concentration of reactant sugars upon heating at 70 and 90 °C.

dissolve, water is more mobile and the system enters a rubbery state, giving increased rate constants (Labuza & Baisier, 1992). The rate of browning decreases again at higher water activities (0.7–0.8), probably due to the increased dilution effect on solute concentration (Eichner & Karel, 1972). This explains the maximum often observed when the rate of non-enzymatic browning reaction is plotted as a function of water activity. In the present study, the levels of a_w were not low enough to clearly confirm the trend of decline in the reaction rate. Returning to the lack of dependence of browning reaction rates on water activity when the initial concentration of reactant sugars was constant at 11.65 °Bx (Fig. 2a and b), this finding indicates the enhanced browning phenomenon in concentrated juice as compared to a diluted system; this can be mainly attributed to the higher initial concentration of sugars rather than the decreased value of water activity, reaching the region where the maximum of browning rates usually occurs. Thus, it is the mass of water rather than the a_w , that should correlate with reaction rate, as reported by Shierwin and Labuza

(2003). In the latter study, the non-enzymatic browning of powders varying in water activity and humectant content (glycerol, sorbitol) was studied. The samples containing sorbitol as humectant gave NEB rates similar to a system free of humectant, whereas samples containing glycerol showed greater reaction rates over the entire a_w and moisture content range studied (0.11–0.75); the responses for the glycerol-containing system were attributed to the higher molecular mobility of this humectant than of sorbitol. Those results were in agreement with the findings of the present study, since apple juice solutions with incorporated sorbitol, at any concentration, showed absorbance evolution (Fig. 2a and b) similar to solutions of the same reactant concentration but without added sorbitol, as produced by dilution with distilled water (curves with diamond patterns in Fig. 2c and d). Thus, despite the strong viscosifying effect of sorbitol, when added in large proportion to the aqueous solution, the overall mobility of reactants in the system is high enough for browning to occur at the same rate.

3.2. Model development

3.2.1. Comparison of the fitting of primary models

Experimental data from a total of 80 absorbance–time curves were fitted to the five primary kinetic models, as given in Section 2.3 (Eqs. (1)–(5)). Previous studies of the non-enzymatic browning have revealed that this reaction fits either zero order (Bozkurt et al., 1999; Burdurlu & Karadeniz, 2003; Ibarz et al., 1999; Koca et al., 2003; Peterson, Tong, Ho, & Welt, 1994; Rattanathanalerk et al., 2005; Shierwin & Labuza, 2003), or first order (Garza et al., 1999; Johnson et al., 1995; Toribio & Lozano, 1984, 1986) kinetic models, with relatively small statistical differences between the two types. A parabolic kinetic model has also been applied by Buedo et al. (2001). Most authors tend to model non-enzymatic browning as a zero order reaction after some induction period, which depends on experimental conditions such as time and temperature (Van Boekel, 2001). By contrast, those who propose first order kinetics for the absorbance variation, claim that the fit of this model improves with increasing reactant concentration (Johnson et al., 1995). However, some researchers have suggested that the true order of non-enzymatic browning reactions may be between zero and one (Nagy et al., 1990; Petriella, Resnik, Lozano, & Chirife, 1985). Thus, since the reaction order of non-enzymatic browning is a debatable issue, this study attempted to obtain complete kinetic information concerning an extended heat treatment of apple juice concentrates by fitting five different kinetic models. The increase of absorbance over time followed a sigmoidal pattern. For many types of growth curves, the growth rate steadily increases to a maximum value before it starts to decline to zero (Seber & Wild, 1989), where a maximum or plateau level for the recorded parameter is reached. This behaviour can be described by the so-called sigmoidal models. Fig. 3 illustrates a graphical comparison of the five kinetic models fitted to a representative absorbance–time plot. It is obvious that the best fits were obtained by the logistic and weibull equations, as given by lines 4 and 3, respectively. These models are the only ones that can incorporate the maximum absorbance (A_{\max}) reached at extended heating times. The physical meaning of A_{\max} reflects a maximum browning potential of juice as a result of reactant solute(s) consumption and/or decomposition of brown pigments formed, progressively leading to no further increases in absorbance. In order to statistically compare the goodness of fit of the five kinetic models to the 80 absorbance–time curves, the difference in residual mean squares (rms = residual sum of squares/degrees of freedom) of all model fittings was used as has been previously done for microbial inactivation kinetic models by Koutsoumanis, Lambropoulou, and Nychas (1999). A graphical comparison of the residual mean square differences between zero and first order models and weibull in pairs with zero, parabolic and logistic models is illustrated in Fig. 4. The maximum browning rate constants, as calcu-

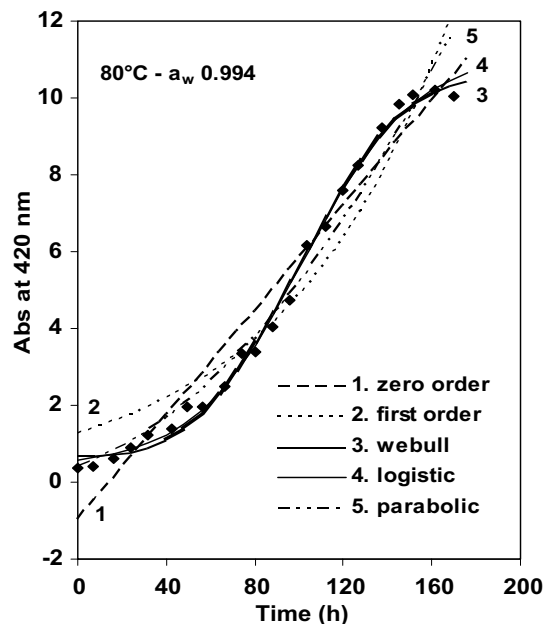


Fig. 3. Graphical comparison of the five kinetic models tested (lines) for fitting the experimental data (symbols) of a respective brown pigment formation curve; apple juice with a_w of 0.99 heated isothermally at 80 °C.

lated by the zero order model (Fig. 4a) and the weibull model (Fig. 4b–d), are presented on the x -axes, respectively. The points below the horizontal axis represent cases where the fit of the first equation type is better than is the second. It is thus clear that the logistic equation had lower residual mean square values in most of the 80 cases examined and thus it was considered as the most appropriate model for describing colour development during thermal processing of the apple juice solutions.

3.2.2. Secondary models describing temperature and water activity dependence of the k and A_{\max} parameters

Since a_w on its own did not significantly affect the NEB of apple juice solutions when the same initial concentration of reactant sugars was present, secondary models describing the temperature and a_w dependence of the k and A_{\max} were developed only for samples diluted with water; these differed in water activity as well as in the initial concentration of reactant sugars. The temperature dependence of browning reaction rates was first modelled by using a modified Arrhenius equation. The parallel positions of the regression lines in the Arrhenius plots (Fig. 5) at all water activity levels, as expressed by a statistically invariant E_a , also led to the conclusion that the temperature dependence of the browning rate is not significantly affected by the a_w . The effect of a_w on browning rate was expressed by the constant d (Eq. (6)), which, in other words, describes an exponential increase in browning rate as the water activity decreases. The proposed modified Arrhenius type model (Eq. (6)) describes both temperature and water activity dependence of browning reaction rate of apple juice concentrates, as shown in Fig. 6a and b, respectively.

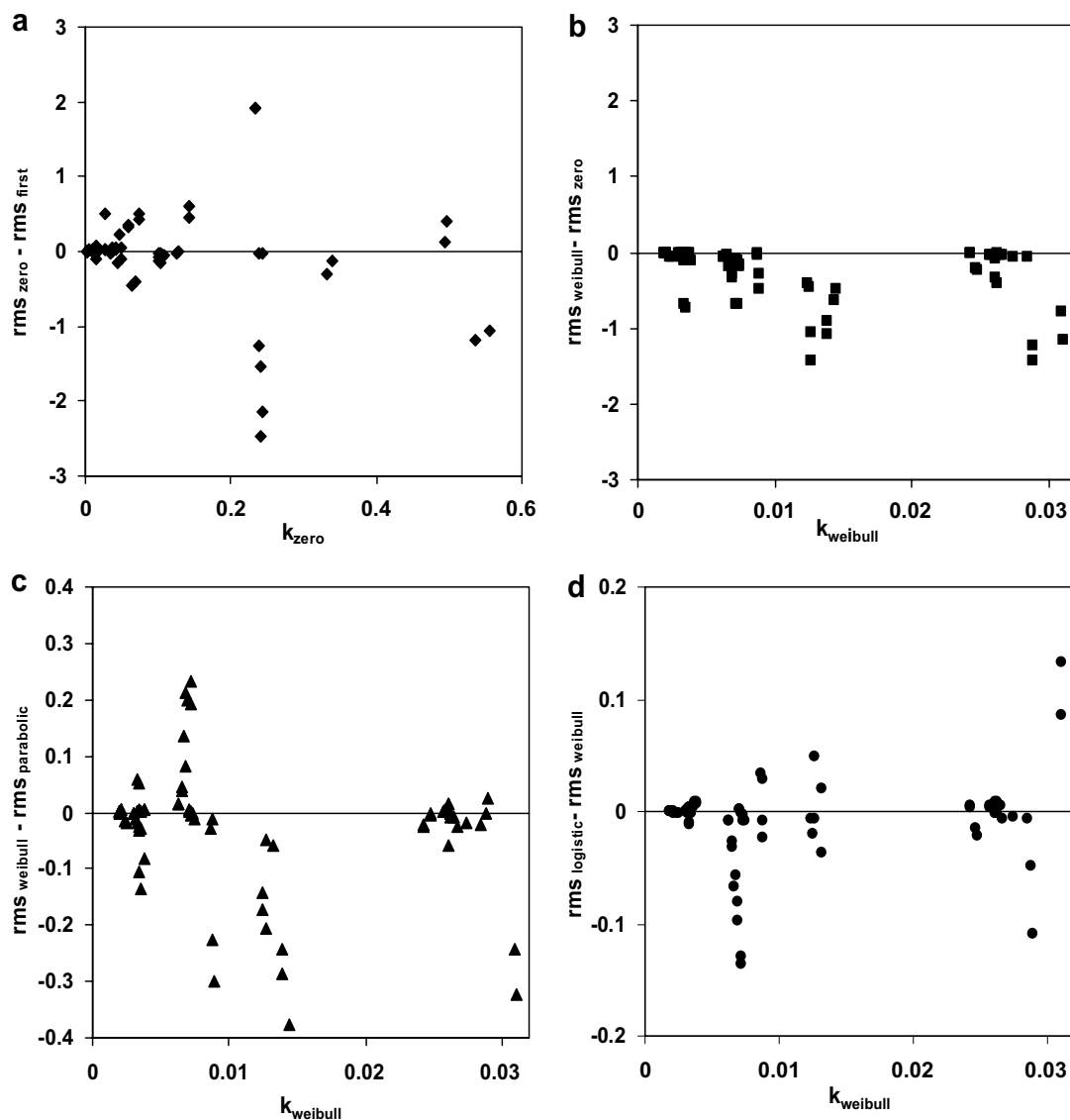


Fig. 4. Comparison of the goodness of fit of the five kinetic models tested, using the difference in the residual mean square, difference between zero and first order kinetic models (a), and comparison of weibull and zero (b), parabolic (c) and logistic models (d). The points below the horizontal axes, represent those cases where the fit of the first model was better than the second (rms difference of y-axis).

In order to describe the combined effect of temperature and a_w on the browning rate of apple juice the parameters of the Arrhenius equation, Belehradek and polynomial models (Eqs. (7)–(9)) were evaluated and are shown in Table 1. Statistics showed lower r^2 values for the Arrhenius than for the polynomial models; the former equation is more theoretically applicable, allowing the expression of the temperature sensitivity of NEB in terms of the estimated E_a (activation energy) parameter and the rate under reference conditions. The application of the Belehradek type of model, revealed that non-enzymatic browning of apple juice concentrates occurs at a projected minimum temperature of 44.4 °C, a value that is likely not realistic. In contrast, the polynomial models gave a slightly improved precision, which was achieved by the addition of one extra parameter beyond those included in the Arrhe-

nus or Belehradek equations. However, none of the four parameters of the quadratic polynomials is interpretable and they do not provide any practical information concerning the temperature dependence of the browning reaction rate.

The estimate of activation energy, 67.0 kJ/mol, as calculated by the modified Arrhenius model (Table 1) for the brown pigment formation of apple juice concentrates, is in the range of 66.9–126 kJ/mol reported in the literature (Labuza & Baiser, 1992). Estimated E_a values of 62.8 and 75.9 kJ/mol were given by Ibarz et al. (1999, 2000) for the A_{420} of thermally treated pear and apple puree, respectively. Manso et al. (2001) reported an E_a of 65.8 kJ/mol for browning of orange juice and Toribio and Lozano (1984) gave E_a values between 69.0 and 83.7 kJ/mol for NEB of an apple juice during storage. Johnson et al.

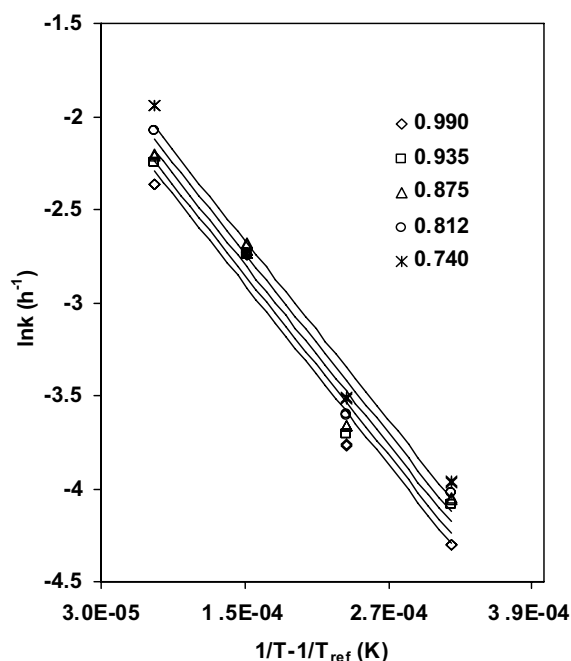


Fig. 5. Arrhenius plots describing the temperature dependence of non-enzymatic browning rate constant (k) of apple juice concentrates differing in a_w .

(1995) have found activation energies of 79.5–105 kJ/mol for browning of orange juice serum, and finally Burdurlu and Karadeniz (2003) gave E_a values in the range of 87.9–141 kJ/mol. However, others have reported higher values, e.g. 116–132 kJ/mol (Bozkurt et al., 1999), 135 and/or 148 kJ/mol (Garza et al., 1999) and 73.6–148 kJ/mol (Koca et al., 2003) for boiled grape juice, peach puree and citrus juice concentrates, respectively. This broad variation in activation energies reported in the literature depends on the specific experimental conditions used and their influence on the reaction mechanism (Van Boekel, 2001).

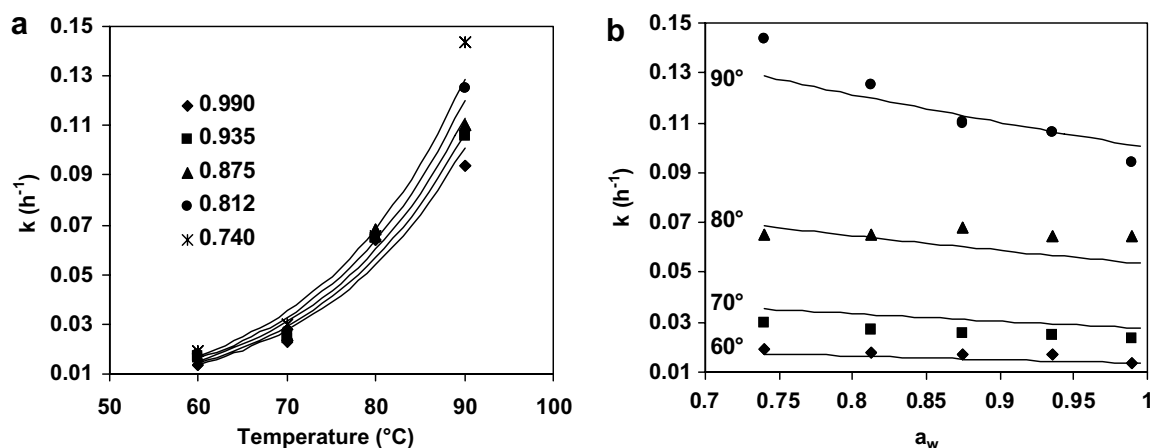


Fig. 6. Effect of temperature (a) and water activity (b) on non-enzymatic browning reaction rate constant; lines in both figures represent the predicted values of rate constants at fixed a_w levels (a) or temperature conditions (b).

Secondary models developed for the parameters expressing the apparent maximum absorbance A_{\max} are presented in Table 2, along with the statistics and estimated coefficients of the model parameters. The polynomial equation, expressing the natural logarithm of A_{\max} as a function of temperature and a_w , was chosen as the most preferable for describing maximum absorbance reached during NEB of apple juice concentrates. The prediction for $\ln A_{\max}$ at various combinations of a_w and processing temperatures is illustrated in Fig. 7. The more concentrated the apple juice (low a_w values) and the higher the temperature, the darker was the final juice, reaching a maximum at the lowest water activity value and the highest temperature tested. Furthermore, in the case of the t_i parameter the quadratic model, describing the temperature and water activity dependence of the natural logarithm of t_i , was the most applicable of the models (data not shown). As was expected, the logistic model parameter A_0 was neither affected by temperature nor by the water activity of any apple juice preparation ($P > 0.05$), since all samples were first diluted to the same solids level (11.65 °Bx) prior to analysis.

3.3. Model validation under isothermal and dynamic heating conditions

The applicability of the derived models was evaluated by comparing the browning rates predicted, using the four different models tested in this study (Eqs. (6)–(9)) and the actual reaction rates observed in independent experiments of isothermal heating (Fig. 8). The Arrhenius model exhibited the smallest difference between the observed and predicted values of reaction rate constants, with most of the predictions falling in the 5% range (Fig. 8a). In contrast, the Belehradec model was less satisfactory, while the polynomial models revealed systematic deviations between the predicted and observed values. Additionally, another comparison of browning rates, as predicted by the Arrhenius

Table 1

Estimated values, confidence limits (CL±, $P < 0.05$) and statistics for the coefficients of four secondary models describing the dependence of browning reaction rate constant (k) on temperature and a_w

Equation type	Parameter	R^2 ^a	Estimated value	CL(±) ^b
Polynomial k	a_1	0.982	$P > 0.05$ ^c	
	a_2		-7.78×10^{-3}	3.21×10^{-3}
	a_3		3.12×10^{-1}	1.41×10^{-1}
	a_4		-4.96×10^{-3}	1.87×10^{-3}
	a_5		1.03×10^{-4}	1.84×10^{-5}
	a_6		$P > 0.05$	
Polynomial \sqrt{k}	a_1	0.982	$P > 0.05$	
	a_2		-7.60×10^{-3}	6.76×10^{-3}
	a_3		2.98×10^{-1}	2.98×10^{-2}
	a_4		-5.44×10^{-3}	3.93×10^{-3}
	a_5		1.30×10^{-4}	3.88×10^{-5}
	a_6		$P > 0.05$	
Behrtradek	b	0.957	7.42×10^{-3}	2.03×10^{-3}
	T_{\min} (°C)		44.36	2.33
	$a_{w \max}$		1.82	5×10^{-1}
Arrhenius	k_{ref} (h^{-1})	0.974	1.81×10^{-1}	7.7×10^{-2}
	E_a (kJ/mol)		66.96	3.68
	d		-9.6×10^{-1}	4.64×10^{-1}

^a Coefficient of determination.

^b ±95% Confidence limit.

^c $P > 0.05$ indicates that the parameter was not significant at the 95% level.

Table 2

Estimated values, confidence limits (CL±) and statistics for the coefficients of three secondary models describing the dependence of A_{\max} on temperature and a_w

Equation type	Parameter	A_{\max}		
		R^2 ^a	Estimated value	CL(±) ^b
Polynomial A_{\max}	a_1	0.935	-400.777	93.3
	a_2		6.05	1.14
	a_3		440.43	179.97
	a_4		-1.97	0.66
	a_5		-2.6×10^{-2}	6.55×10^{-3}
	a_6		-191.82	99.90
Polynomial $\ln(A_{\max})$	a_1	0.978	-39.52	5.66
	a_2		6.7×10^{-1}	6.9×10^{-2}
	a_3		39.21	10.92
	a_4		-8.62×10^{-2}	4.03×10^{-2}
	a_5		-3.61×10^{-3}	3.97×10^{-3}
	a_6		-20.94	6.06
Arrhenius	$A_{\max \text{ ref}}$	0.731	20.37	3.81
	E_a (kJ/mol)		52.09	11.93
	d		-3.53	1.51

^a Coefficient of determination.

^b ±95% Confidence limit.

model, and the observed reaction rates for all conducted experiments, revealed a satisfactory agreement by the spread of all data points close to the diagonal (Fig. 9). Thus, the modified Arrhenius model was proved to be the most applicable for describing the temperature and

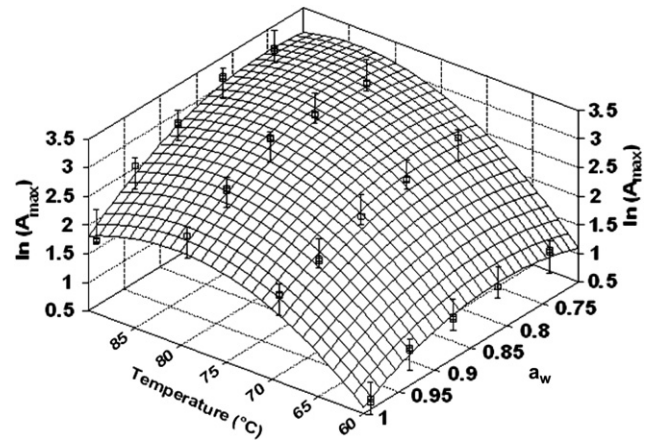


Fig. 7. Prediction surface plot showing the plateau absorbance, A_{\max} , as affected by temperature and a_w ; points and bars represent prediction and 95% prediction intervals, respectively, of the response $\ln(A_{\max})$ for a given temperature and a_w combination.

water activity dependence of non-enzymatic browning reaction rate.

In the food industry, thermal processing in the production chain usually involves varying time–temperature protocols which significantly affect the final quality characteristics of the food products. In order to better predict the quality deterioration of apple juice concentrates, all the previously discussed kinetic modelling was employed to monitor the absorbance evolution of NEB under non-isothermal heating conditions. In the field of predictive microbiology, an accepted relevant approach used to predict the microbial growth under fluctuating temperature scenarios is to divide the time–temperature history into short assumed constant temperature/time intervals (Fu & Labuza, 1993; Koutsoumanis, 2001). In this context, the absorbance evolution of apple juice concentrates at fluctuating temperatures was predicted using the method described by Koutsoumanis (2001) which was modified as follows:

At time $t = dt_1$,

$$A_{t1} = A_{\max t1} / \left(1 + \left(\frac{A_{\max t1}}{A_0} - 1 \right) \exp(-k_1 dt_1) \right)$$

At the subsequent time interval, $t = dt_2$

$$A_{t2} = A_{\max t2} / \left(1 + \left(\frac{A_{\max t2}}{A_{t1}} - 1 \right) \exp(-k_2 dt_2) \right)$$

and at time $t = dt_i$

$$A_{ti} = A_{\max ti} / \left(1 + \left(\frac{A_{\max ti}}{A_{t(i-1)}} - 1 \right) \exp(-k_i dt_i) \right) \quad (10)$$

Additionally the following conditions were assumed:

$$A_{ti} = \begin{cases} A_{ti} & \text{if } A_{\max ti} > A_{t(i-1)} \\ A_{t(i-1)} & \text{if } A_{\max ti} < A_{t(i-1)} \end{cases}$$

where dt_i ($i = 1, 2, 3$, etc.) is a short assumed constant temperature time interval, A_0 is the initial absorbance

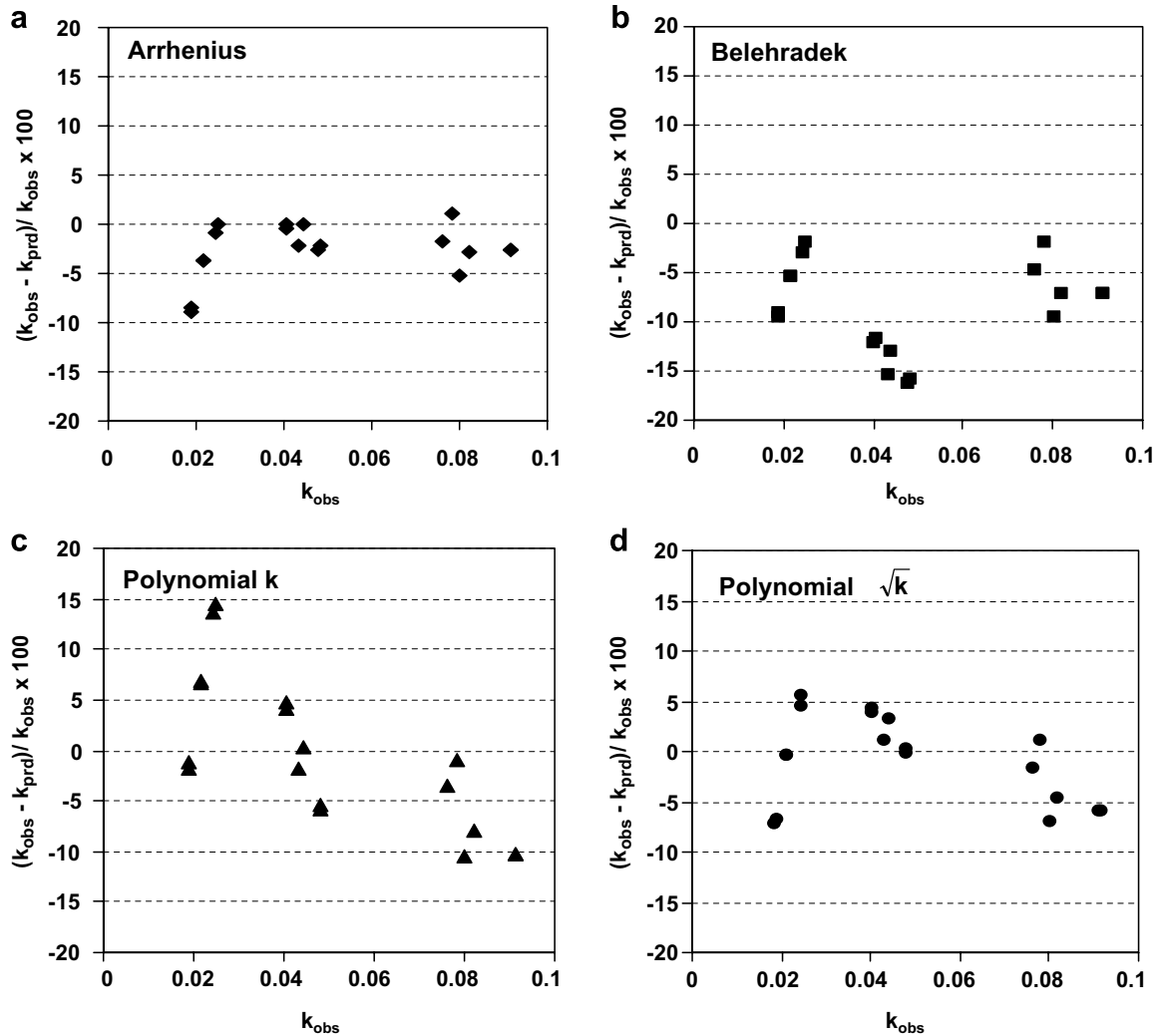


Fig. 8. Percent deviations between the observed (k_{obs}) in the independent experiments and predicted (k_{prd}) values of browning rates (k) of apple juice as estimated with the four tested secondary models.

measurement at time zero, A_{ti} is the absorbance estimation at a time interval dt_i , $A_{\text{max } ti}$ is the maximum absorbance value calculated by the secondary model at the temperature corresponding to the time interval dt_i , and k_i is the reaction rate constant at the temperature during dt_i , as estimated by the modified Arrhenius equation.

The ability of the derived dynamic model to predict non-enzymatic browning of apple juice concentrates under non-isothermal heating conditions, was evaluated by comparing the predicted absorbance evolution curves (Eq. (10)) to actual browning data obtained from four experiments involving fluctuations of storage temperatures. At first, two prolonged heating temperature profile scenarios of periodic temperature changes were studied, where the apple juice concentrate was heated for 12 h at 65 °C and 12 h at 85 °C (Fig. 10a) or 12 h at 65, 6 h at 75 and 6 h at 85 °C (Fig. 10b). In both cases the reconstructed dynamic logistic model was able to predict in good agreement, the absorbance evolution of NEB of apple juice concentrate for the non-isothermal heating protocol. The last two proto-

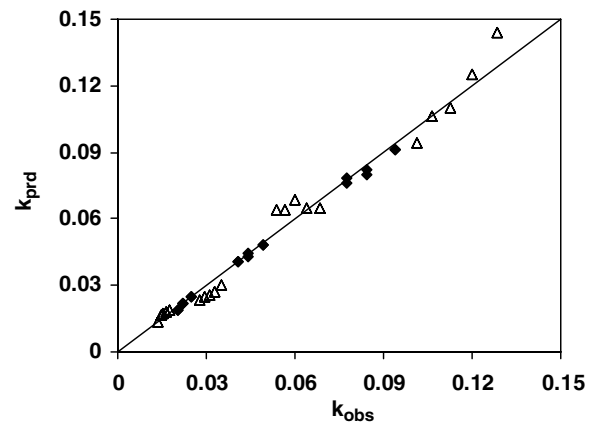


Fig. 9. Plot of predicted (k_{prd}) (using Arrhenius model) versus observed (k_{obs}) values of apple juice browning reaction rates heated under various combinations of temperature and a_w ; open symbols represent browning rate constants obtained in the first stage of the experimental design assuming isothermal heating conditions and the closed symbols those of independent experiments.

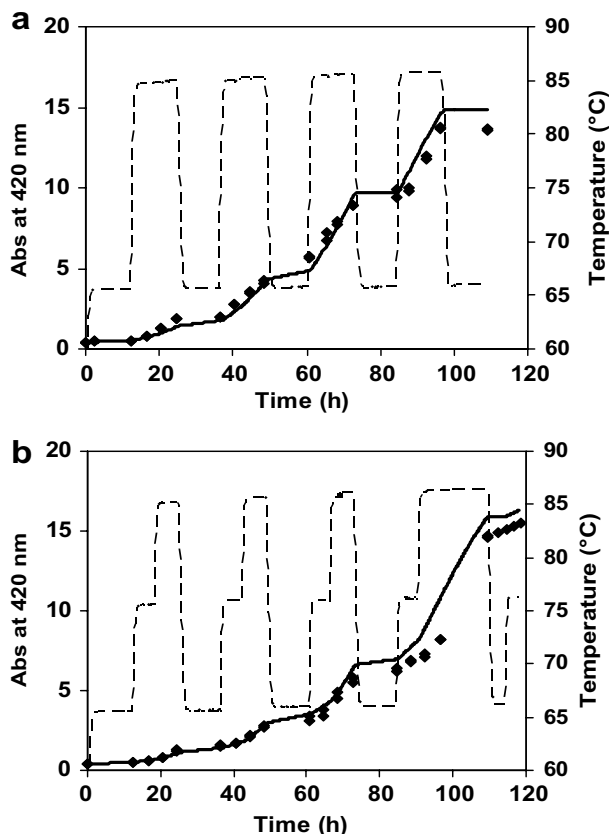


Fig. 10. Comparison between observed (points) and predicted (line) browning of apple juice (a_w 0.865) heated under periodically changing temperature profiles for 12 h at 65 and 12 h at 85 °C (a) and 12 h at 65, 6 h at 75 and 6 h at 85 °C (b); dotted lines depict the temperature fluctuations.

cols involved thermal treatment of apple juice for either 2 h at 65 °C, 2 h at 75 °C and 2 h at 85 °C (Fig. 11a) or 3 h at 65 °C and 3 h at 85 °C (Fig. 11b) (total heating time of 6 h). A fairly good agreement between the actual browning values and those predicted by the model was also evident in the short time dynamic heating protocol, indicating that prediction of non-enzymatic browning is also possible in thermal treatments resembling the actual pasteurization process.

4. Conclusions

The main contribution of this paper is a complete kinetic assessment of the non-enzymatic browning phenomenon of apple juice concentrates under both isothermal and dynamic heating conditions, where the individual and combined effects of temperature and water activity have been investigated. The processing temperature had a strong impact on browning kinetics for juice concentrates having both the same and different initial concentration of reactant sugars. The initial reactant concentration, and not the a_w itself, seems to have a significant effect on colour development. Modelling of the kinetic data revealed that the logistic model was the most appropriate for describing

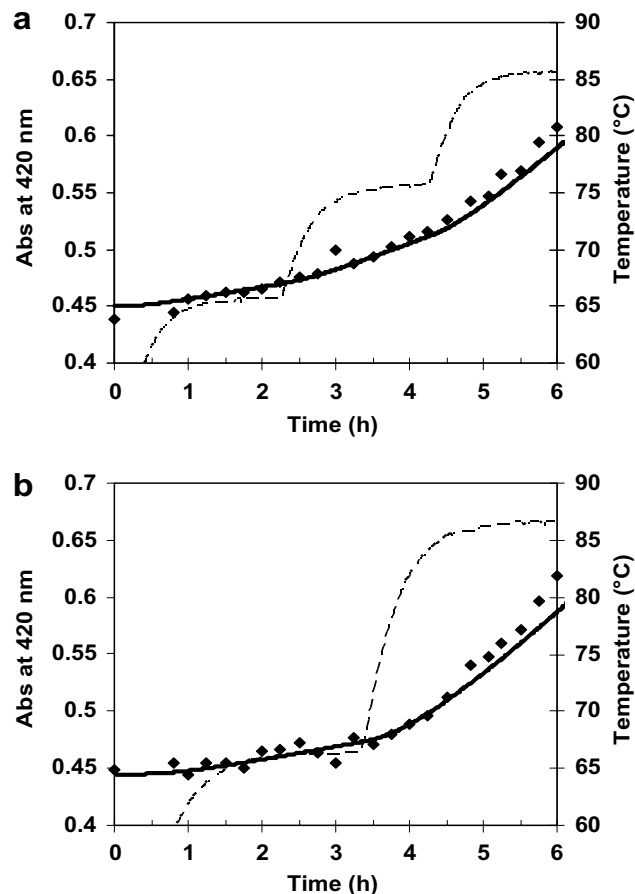


Fig. 11. Comparison between observed (points) and predicted (line) browning of apple juice (a_w 0.74) heated for 2 h at 65, 2 h at 75 and 2 h at 85 °C (a) and for 3 h at 65 and 3 h at 85 °C (b); dotted lines depict the temperature fluctuations.

colour development in apple juice concentrates. Moreover, the applied modified Arrhenius equation for the dependence of browning reaction rate on temperature and a_w was suitable for predicting the rate constant, providing values in close agreement with the observed reaction rates in independent experiments. The overall derived dynamic logistic model satisfactorily predicted NEB of thermally processed apple juice with dynamic heating protocols.

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