2010-01-01

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Recommended Citation

P.J. Cullen, et al. (2010). Quantitative modelling approaches for ascorbic acid degradation and non-enzymatic browning of orange juice during ultrasound processing. *Journal of Food Engineering,* Vol. 96(3), 449-454. doi:10.1016/j.jfoodeng.2009.08.025

Quantitative modelling approaches for ascorbic acid degradation and non-enzymatic browning of orange juice during ultrasound processing

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Quantitative modelling approaches for ascorbic acid degradation and non-enzymatic browning of orange juice during ultrasound processing

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Abstract

The objective of this study was to develop a deterministic modelling approach for non-enzymatic browning (NEB) and ascorbic acid (AA) degradation in orange juice during ultrasound processing. Freshly squeezed orange juice was sonicated using a 1,500 W ultrasonic processor at a constant frequency of 20 kHz and processing variables of amplitude level (24.4 – 61.0 μm), temperature (5 – 30 ^{o}C) and time (0 – 10 min). The rate constants of the NEB and AA were estimated by a primary model (zero and first order) while their relationship with respect to the processing factors was tested for a number of models, i.e., second order polynomial, different types of Ratkowsky-type model, and an Arrhenius-type model. The non-monotonic behaviour of NEB has been described more accurately by the use of a polynomial model. The rate constants of AA were described by a similar type of model having a monotonic behaviour. A synergistic effect of temperature for different amplitudes on the rate constant of both NEB and AA was observed, while an antagonistic effect of amplitude on the rate of NEB was evident. The models with the best fit were integrated to produce contour plots for the combined amplitude and temperature. The constructed contour plots illustrate that low temperatures and intermediate amplitudes, i.e., 42.7 μm, result in lower NEB and AA deterioration and consequently better quality orange juice. The overall developed modeling approaches exploit quality data in order to identify the optimal processing regions for eliminating quality deterioration of orange juice during ultrasound processing which is of high importance to the food industry.

Keywords: Ultrasound, Ascorbic acid, non-enzymatic browning, modelling

Introduction

The use of ultrasound within the food industry has been a subject of research and development for many years with applications in both food analysis (diagnostic ultrasound) and food processing (power ultrasound). Power ultrasound has been recognized as a promising processing technology to replace or complement conventional thermal treatment in the food industry. When high power ultrasound propagates in a liquid, cavitation bubbles are generated due to pressure changes. These micro bubbles collapse violently in the succeeding compression cycles of a propagated sonic wave resulting in localised high temperatures up to 5000 K, pressures up to 50,000 kPa, and high shearing effects (Suslick, 1988). Consequently, the intense local energy and high pressure bring a localised pasteurisation effect. Advantages of sonication include reduced processing time, higher throughput and lower energy consumption while reducing thermal effects (Zenker et al. 2003; Knorr et al. 2004). Various research groups have demonstrated the inactivation of pathogenic and spoilage microorganisms (*Escherichia coli*, *Listeria*), spoilage enzymes (pectin methyl estrase, polyphenol oxidase) with reduced effects on quality or nutritional parameters including ascorbic acid in orange juice (Tiwari et al. 2008a), ascorbic acid and anthocyanins content in strawberry (Tiwari et al. 2008b) and blackberry juice (Tiwari et al. 2009b). Power ultrasound has been employed for inactivation of *E. coli* in apple cider (Baumann et al. 2005) and orange juice processing (Valero et al. 2007; Tiwari et al. 2008a). Similarly, enzymes such as peroxidase (De Gennaro et al. 1999), proteases and lipases (Vercet et al. 2001) were reported to be inactivated.

The nutritional quality of orange juice is primarily related to the ascorbic acid content (Zerdin et al. 2003). Ascorbic acid is thermolabile and highly sensitive to various processing conditions. The mechanism of vitamin C degradation follows aerobic and/or anaerobic pathways and depends upon several processing conditions (Tannenbaum 1976; Vieira et al. 2000). Ultrasound treatment of orange juices is reported to have a minimal effect on the ascorbic acid content during processing and results in improved stability during storage when compared to thermal treatment (Tiwari et al. 2009a). This positive effect of ultrasound is assumed to be due to the effective removal of occluded oxygen from the juice (Knorr et al. 2004), a critical parameter influencing the stability of ascorbic acid (Solomon et al. 1995). Tiwari et al. (2009a) reported a maximum degradation of 5 % in the ascorbic acid content of orange juice when sonicated at the highest acoustic energy density (0.81 W/mL) and treatment time (10 min). During storage at 10 ^{o}C sonicated juice was found to have a higher retention of ascorbic acid compared to thermally processed and control samples. Several studies have shown that non-thermal process technologies including high pressure, pulsed electric fields and sonication retain a higher level of ascorbic acid relative to thermally processed juices (Yeom et al. 2000; Torregrosa et al. 2006; Cheng et al. 2007; Tiwari et al. 2009a). Non-enzymatic browning (NEB) significantly influences the commercial value of citrus products, as it is the first visible quality defect to be detected during ambient temperature storage. In citrus juices, NEB may result from reactions of sugars, amino acids and ascorbic acid.

Kinetic models can be used for objective, fast and economic assessments of food quality. Kinetic modeling may also be employed to predict the influence of processing on critical quality parameters. The objective of this study was to develop integrated deterministic modeling approaches of both quality indices, i.e., AA and NEB, to identify the optimal processing conditions for producing orange juice with minimal quality deterioration. Therefore, the kinetics of the quality indices of NEB and AA are described quantitatively in order to evaluate the combined effect of the extrinsic parameters of amplitude and temperature on them. The developed deterministic modeling approaches of both quality indices are integrated in order to identify the optimal conditions for producing an orange juice with minimal quality deterioration.

Materials and methods

Juice preparation

Oranges (*Citrus sinensis cv. Valencia*) were purchased from a local fruit supplier (Reilly Wholesale Ltd., Dublin Ireland). Fresh juice was squeezed using a household table top citrus juice extractor (BRAUN Gmbh, Kronberg, Germany) and filtered using a double layer cheese cloth to remove pulp. Orange juice extraction and filtration were performed in a cold room maintained at 3 ±1 °C. Juice obtained was immediately frozen at -25 ^{o}C. Frozen juice samples were processed within one month of juice preparation.

Ultrasound treatment

A 1,500 W ultrasonic processor (VC 1500, Sonics and Materials Inc., Newtown, USA) with a 19 mm probe was used for sonication (Fig. 1). Samples were processed at a constant frequency of 20 kHz. The energy input was controlled by setting the amplitude of the sonicator probe. Extrinsic parameters of temperature (5, 10, 15, 20, 25, 30 ^{o}C), amplitude (24.4, 42.7, 61.0 µm) and treatment time (2, 4, 6, 8, 10 min) were varied with pulse durations of 5 s on and 5 s off. Eighty mL orange juice samples were placed in a 100 mL jacketed vessel through which water at a flowrate of 0.5 L/min was circulated (Fig. 1). Sonication at the desired amplitude level was started once the set temperature was reached in the jacketed beaker. The ultrasound probe was submerged to a depth of 25 mm in the sample. All treatments were carried out in triplicate.

Determination of non-enzymatic browning

Non-enzymatic browning was measured using the method of Meydav et al. (1977). Ten mL orange juice samples were centrifuged for 10 min; 756 *g* and 20 ± 0.5 °C (Sigma 1A, AGB Scientific Ltd, Dublin, Ireland) to remove coarse particles. Five mL of ethyl alcohol (95%, Sigma-aldrich, Dublin, Ireland) was added to 5 mL of juice supernatant and centrifuged as above. The absorbance of the supernatant was obtained at 420 nm using a Unicam UV-VIS (UV2) spectrophotometer with distilled water as blank. Measurements were taken in triplicate and mean value reported. s

Determination of ascorbic acid

Ascorbic acid content was determined following the HPLC (Shimadzu Model no: SPD - M10AVP, Shimadzu Co., Japan) analytical procedure outlined by Lee and Coates (1999). To prepare the sample, 25 mL of the juice samples were added into 50 mL centrifuge tubes containing 5 mL of 2.5% metaphosphoric acid. Samples were centrifuged for 10 min; 2000 *g* and 4 ^{o}C. Then, 5 mL of the supernatant was filtered through PTFE syringe filters (0.45µm, Phenomenex, U.K) and placed in an autosampler vial. Ten µL aliquot of samples were injected onto a Shimadzu C18 (15cm × 4.6cm, pore size 5μm) coupled with HyperODS guard column. The mobile phase was 25 mM KH_2PO_4 (adjusted to pH 3.0 with phosphoric acid) at a flow rate of 1 mL/min. Eluate was monitored by UV detection at 245 nm. Chromatograms were recorded and processed with EZStart Chromatography Software V.7.2.1. Results were reported as g of ascorbic acid/L of orange juice.

Overall experimental design

A general factorial design (SAS V.9.1, SAS Institute, NC, USA) consisting of 180 experimental trials (including the 3 replicates) was employed. During ultrasound treatment, the effects of amplitude (μm), temperature (^oC) and treatment time (min) were studied. Analysis of variance (ANOVA) was carried out to determine any significant differences (*P < 0.05*) among the applied treatments.

Model development

The rate constants for NEB and AA were estimated by a primary model describing the evolution of the concentration of a component, i.e., NEB and AA, with respect to the time. A zero order and a first order model were employed for this purpose:

$$C(t) = C(0) + k \cdot t \qquad (1)$$

$$C(t) = C(0) \cdot \exp(k \cdot t) \qquad (2)$$

where *C*(t) represents the AA concentration [mg/ 100 mL of orange juice] and the NEB level respectively, at time *t* and *k* is the rate constant. The relationship of the rate constant, *k*, with respect to the processing factors was tested for a number of secondary models, i.e., second order polynomial, different types of Ratkowsky-type model, and an Arrhenius-type model. The second-order response surface model with an interaction factor is expressed as:

$$k = \beta_o + \beta_1 \cdot T + \beta_2 \cdot T^2 + \beta_3 \cdot A + \beta_4 \cdot A^2 + \beta_5 \cdot T \cdot A \qquad (3)$$

where β_i are the polynomial coefficients and T and A are the temperature [^{o}C] and amplitude levels [μm], respectively. Only significant parameters (P<0.05) were retained by performing a stepwise fit. An Arrhenius type equation inspired by the model of Cerf (Cerf et al. 1996) was developed, in which the effect of the temperature and amplitude on the rate constants of NEB and ascorbic acid was investigated. The model is:

$$k = C_o + C_1 \cdot A^2 + \frac{C_2}{T} \qquad (4)$$

Where C_o, C_1 and C_2 are the coefficients of the Arrhenius type model. This type of model correlates the rate constants against the reciprocal temperature to produce a mathematical structure having an Arrhenius format.

Two different types of the Ratkowsky type models (Ratkowsky et al. 1983) have also been considered. For these equations the squared root of the rate constant has been considered aiming at the stabilisation of the variance of the rate constants. These transformed equations appear as follows:

$$\sqrt{k} = \alpha_1 \cdot (A + \alpha_2) \cdot (T + \alpha_3) \qquad (5)$$

$$\sqrt{k} = \alpha_1 \cdot (A + \alpha_2)^2 \cdot (T + \alpha_3) \qquad (6)$$

When Eq. (6) is compared with Eq. (5) it can be observed that the second factor of the right hand side of the equation has been adjusted such as to evaluate a quadratic effect of amplitude changes on the rate constants.

In case of the kinetics of NEB the following equation has also been employed:

$$\sqrt{k} = \alpha_1 \cdot (A + \alpha_2) \cdot (1 + \exp(\alpha_3 \cdot (\alpha_4 - A)) \cdot (T + \alpha_5)) \qquad (7)$$

Where α_i are the coefficents of determination for these models. Observe that Eq. (7) has been transformed in such a way that could take into account the antagonistic effect of amplitude at different temperatures on the non-enzymatic rate constants (see constant rates of NEB in Fig. 2).

The different secondary models are evaluated with respect to their performance and the best fitted models are used to construct iso-rate contour plots that integrate both NEB and AA kinetics for the combined amplitude and temperature treatments. The iso-rate contour plots are further exploited for process optimisation.

Statistical analysis

Only significant parameters have been retained for the tested models (P<0.05). For the evaluation of the fitting capacity of the models the statistical criterion of the adjusted coefficient of multiple determination R^2_{adj} and the root mean squared error *RMSE* have been used.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n_t} (y_{\exp}(t_i) - y(t_i, p_{ls}))^2}{n_t - n_p}} \qquad (8)$$

Where $y_{exp}(t_i)$ denotes the experimental observations, $y(t_i,p_{ls})$ the predicted values, n_t the total number of data points, n_p the number of estimated model parameters.

$$R^2{}_{adj} = 1 - \left(\frac{n_t - 1}{n_t - n_p} \right) \cdot \frac{SSE}{SSTO} \qquad (9)$$

Herein, *SSTO* is the total sum of squared errors $\sum (y_i - \bar{y})^2$ and SSE the sum of squared errors $\sum (y_{\exp}(t_i) - y(t_i, p_{ls}))$.

Software Programs

For simulation, optimisation, and fitting of the data, programs were written in MatLab® Version 6.5 (The MathWorks, MA, USA). The optimisation routines employed were *lsqnonlin* (for the Ratkowsky type models) and *lsqlin* (MatLab Optimization Toolbox). The *stepwisefit* routine was employed for stepwise regression (MatLab Statistics Toolbox) .

Results & Discussion

Non-enzymatic browning (NEB)

The NEB index followed a zero order reaction Eq (1) with respect to treatment time for the different amplitudes and temperatures studied. Previous kinetics studies on browning index reactions based on A420 nm measurement in citrus juices, apple juices (Burdurlu and Karadeniz 2003), pear puree (Ibarz et al. 1999) and pear juice concentrate (Beveridge and Harrison 1984) similarly reported zero-order reaction kinetics. The estimated parameters of rate constants *k*, for each replicated study are illustrated in Fig. 2. Eq. (7) of the modified Ratkowsky model described the observed non-linearities (R^2_{adj} = 0.975, *RMSE* = 0.0031) better than Eqs. (5, 6), but resulted in non-accurate parameters, i.e., SE errors were much higher than the estimated parameters. This may be attributed to the limited amount of data describing the antagonistic behaviour of amplitude on NEB. Among all the secondary models tested, the polynomial model (Eq. (3)) gave the best regression performance for describing the non-monotonic behaviour of the effect of amplitude on the NEB constants (Fig. 2). All its parameters appeared to be significant ($P<0.05$) (Table 1).

The lower P-values (so the more 'significant' the results) were obtained for the coefficient of β_4 (P=0) (quadratic effect of Amplitude) and β_2 (P=$1.08\text{x}10^{-26}$) (linear effect of temperature) indicating that for the same temperature levels the NEB rate constants were highly dependent on the amplitude levels followed by the temperature levels. Interactive effects gave higher P values ($1.36\text{x}10^{-6}$). The NEB rate significantly increased with processing temperature while at intermediate ultrasound amplitudes appeared to have lower values indicating an antagonistic effect of amplitude on the rate of NEB. More specifically, at amplitude levels of the range of 42.7 μm, the NEB rate appeared to be lower than at higher or lower amplitude levels for the same temperatures. The observed monotonic increase of the NEB rate with respect to temperature has also been reported for the browning kinetics of apple juice and apple cider (Ugarte-Romero et al. 2006; Vaikousi et al. 2008). Nonenzymatic browning may result from the condensation of a carbonyl group with amino acids, reactions of sugars and ascorbic acid in the absence of free amino acids (caramelization). The obtained increase of the browning rate at high amplitudes can be attributed to the decrease of sugar content (Yuan et al. 2009).

Ascorbic acid degradation (AA)

A significant ($p<0.05$) reduction in orange juice ascorbic acid content (mg/100 mL) was observed as a function of treatment time. The degradation kinetics of ascorbic acid followed first order kinetics (Eq. (2)) and the estimated rate constants for each of the replicates are illustrated in Fig. 3. Similar kinetic behaviour on watercress processed by thermosonication was observed by other authors (Cruz et al. 2008).

The largest AA reduction was observed at the highest amplitude (61.0 μm) and processing temperature (30 °C), . However this reduction was less than 15% loss of the initial ascorbic acid content of the unprocessed juice. The ascorbic acid rate constant with respect to the amplitude and the temperature was described more accurately by employing the polynomial model (Eq. (3)) (Table 2).

The lower P-values were obtained for the coefficient of β_3 (P=0) (linear effect of amplitude) followed by β_5 ($P=6.16\text{x}10^{-8}$) (interactive effect of amplitude and temperature). Fig. 3 illustrates that increase of temperature and increase of amplitude resulted in higher ascorbic acid loss. This indicates a synergistic effect of temperature for different amplitudes and temperatures on the AA rate constant.

Several mechanisms can act concurrently when ultrasound is applied in liquid systems, i.e., thermal effects produced by bubble implosion, mechanical stresses produced microstreaming and implosion shock waves, and free radical production. Nevertheless, radical productions have been considered the most probable mechanism (Portenlanger and Heusinger 1992; Vercet et al. 2001). The degradation of ascorbic acid divides into two sections corresponding to aerobic and anaerobic degradation (Nagy 1980; Eisonperchonok and Downes 1982; Robertson and Samaniego 1986; Kennedy et al. 1992; Ariahu et al. 1997; Blasco et al. 2004). Sonication results in a reduction of dissolved oxygen, a critical parameter influencing the stability of ascorbic acid (Solomon et al. 1995). Hydroxyl radical formation is found to increase with degassing. Sonication cavities can be filled with water vapour and gases dissolved in the juice such as O_2 and

N_2 (Korn et al. 2002). The interactions between free radicals and ascorbic acid may occur at the gas–liquid interfaces. In summary ascorbic acid degradation may follow one or both of the following pathways:

Ascorbic acid $\rightarrow$ thermolysis (inside bubbles) and triggering of Maillard reaction

Ascorbic acid $\rightarrow$ reaction with OH^- $\rightarrow$ HC–OH and production of oxidative products on the surface of bubbles

Thus sonication can be related to advanced oxidative processes since both pathways are associated with the production and use of hydroxyl radicals (Petrier et al. 2007). Previous publications have shown that vitamin C degradation in different type of processes was following first order kinetics independently of the pathway followed (Nisha et al. 2004; Vikram et al. 2005).

Contour design

An analysis of amplitude and temperature diagrams was performed based on the best fitted mathematical expressions (see previous Sections). Iso-rate contour plots integrating NEB and AA information were developed (Fig. 4). The constructed contour plots illustrate that low temperatures and intermediate amplitudes, i.e., 42.7 μm, result in lower NEB and AA deterioration and consequently better quality of orange juice. Non enzymatic browning effects appear to be more sensitive to ultrasound processing than ascorbic acid degradation (Fig. 4). Based on the obtained contour plots is suggested that NEB could be more appropriate to determine the intensity of an ultrasound processing during commercial applications. This is in line with the fact that browning reactions of ascorbic acid are among the browning indexes while measuring the overall NEB effects. Nevertheless the importance of using the ascorbic acid as a quality and shelf life indicator of orange juice in the juice processing is evident and will have to be considered for performing additional shelf life studies.

Conclusion and future work

The modeling approaches developed in this study exploit data in order to identify the optimal processing regions for eliminating quality deterioration of orange juice during ultrasound processing which is of high importance in food industry. The non-monotonic behaviour of NEB has been described more accurately by the use of a polynomial model. The rate constants of AA were described by a similar type of model having a monotonic behaviour. A synergistic effect of temperature for different amplitudes on the rate constant of both NEB and AA was observed, while an antagonistic effect of amplitude on the rate of NEB was evident. Ultrasound was found to have more drastic effect on NEB than AA degradation of orange juice.

The implemented modelling approaches could be further developed for incorporating a prior knowledge of the kinetic process during the parameter estimation as this approach was previously suggested and applied (Geeraerd et al. 2004; Valdramidis et al. 2007). This would require the collection of additional biochemical information on the browning and ascorbic acid dynamics of different juice products during ultrasound processing. Consequently mathematical terms under the form of partial derivatives that describe monotonic or non-monotonic quality kinetics can be developed and used for the parameter estimation of the suggested model structures. Additional biochemical studies e.g., enzymatic browning, formation of browned polymers, may also be exploited to carry out multi-objective optimisations of fruit juice processing aimed at the production of high quality sonicated fruit products..

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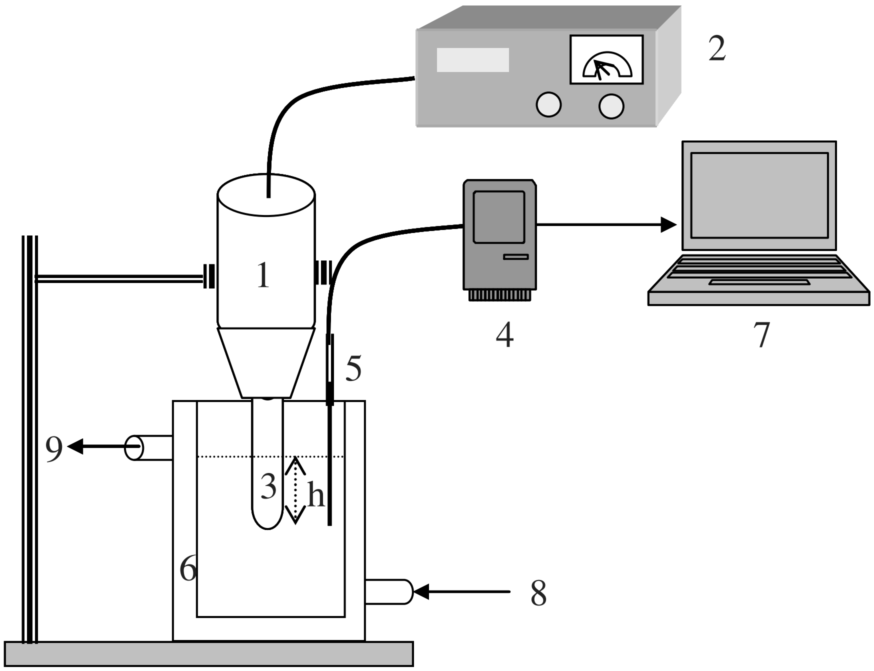

Figure 1. Experimental setup (1) ultrasound transducer; (2) ultrasonic generator; (3) ultrasound probe (19 mm); (4) data logger; (5) temperature probe; (6) jacketed beaker; (7) computer; (8) water inlet; (9) water outlet; (h) depth of probe in to the sample (25 mm)

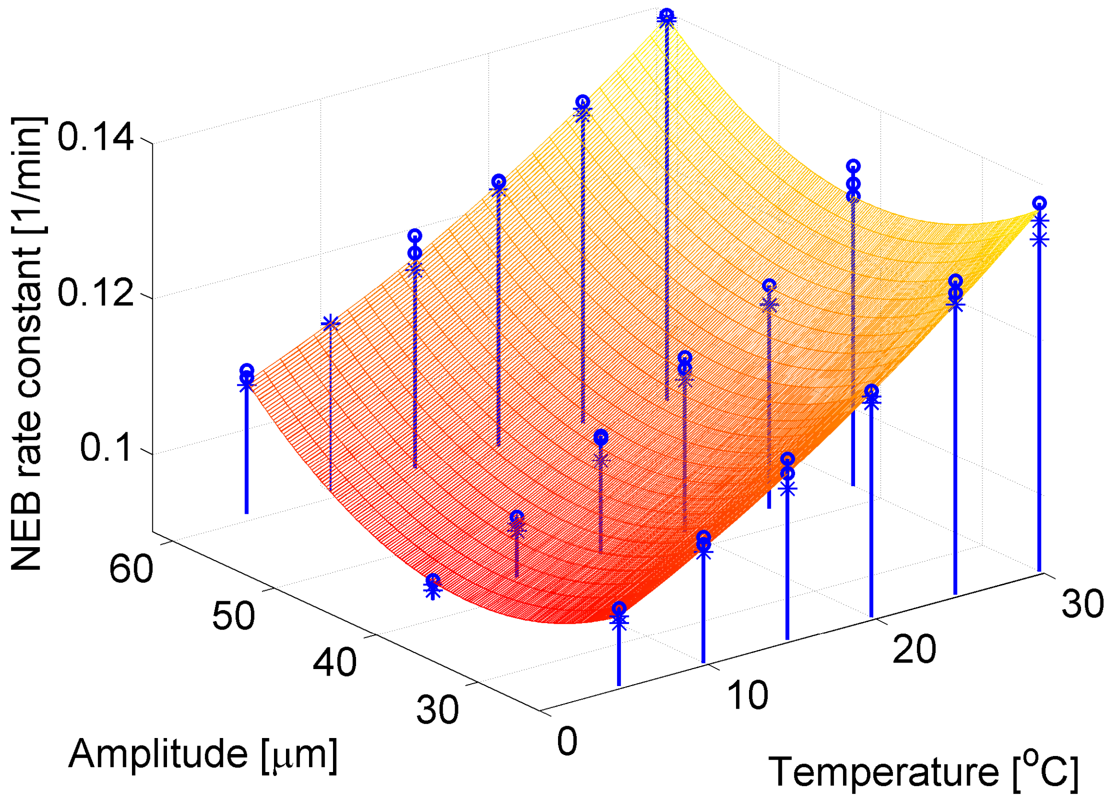

Figure 2. Modelling the non enzymatic browning rate constant, *k* (Eq. (3), Table 1). (o): experimental data points above the surface, (*): experimental data points under the surface.

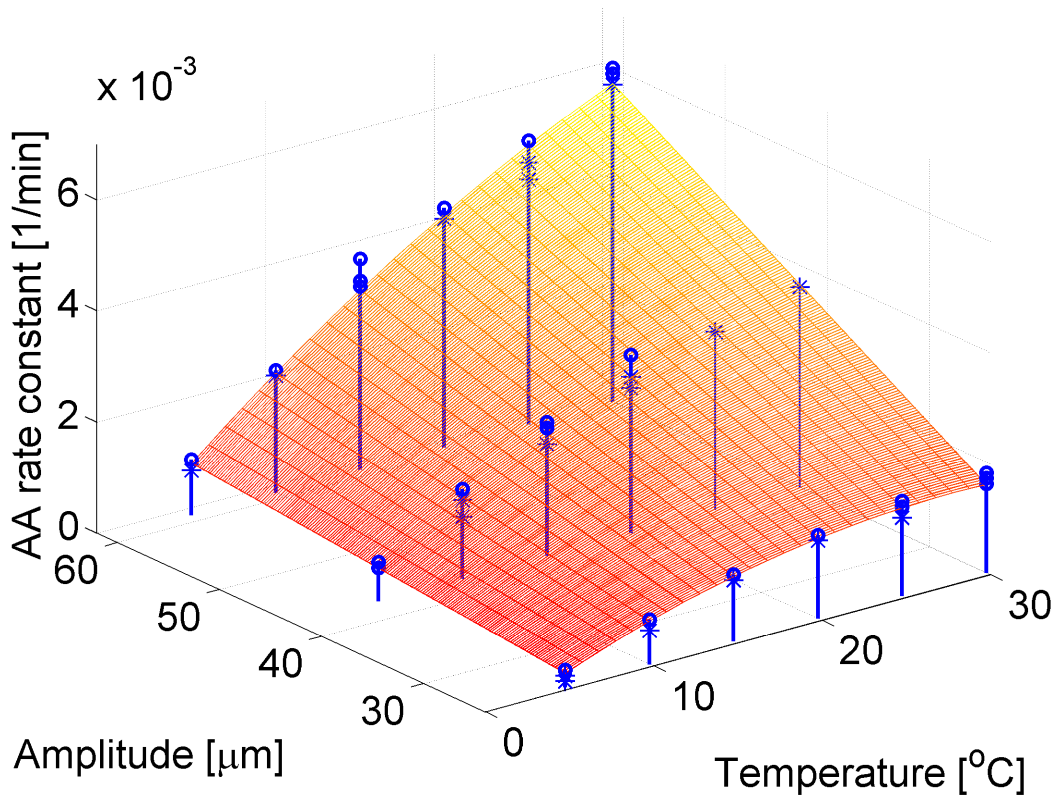

Figure 3. Modelling the ascorbic acid rate constant, k (Eq. (3), Table 2). (o): experimental data points above the surface, (*): experimental data points under the surface.

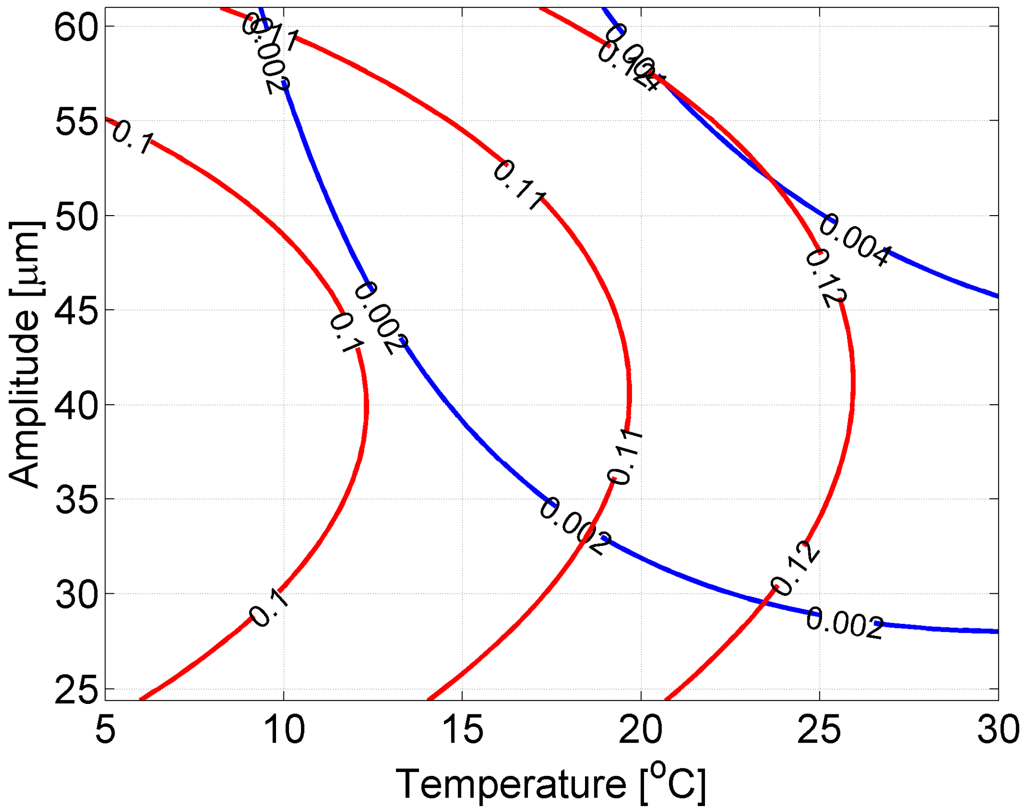

Figure 4. Non enzymatic browning and ascorbic acid iso-rate contour plots for $k = 0.1$, 0.11, 0.12 [1/min] and $k = 0.004$, 0.004 [1/min], respectively.

Table 1. Results on the parameter estimates of the secondary fitted models (Eqs. (3)- (6)) for the NEB kinetics.

Equation type	Parameters			
		R^2_{adj}, *RMSE*	Estimated values	SE
Polynomial (Eq. (3))	β_o	0.998, 0.0015	0.135	0.003
	β_1		-2.500	1.127×10^{-4}
	B_2		1.000	1.331×10^{-4}
	β_3		-5.964	1.571×10^{-6}
	β_4		3.193	1.270×10^{-6}
	β_5		1.773	3.214×10^{-6}
Arrhenius type (Eq. (4))	C_o	0.6187, 0.072	-2.078	0.023
	C_1		1.927×10^{-5}	7.649×10^{-6}
	C_2		-1.553	0.172
Ratkowsky type (Eq. (5))	α_1	0.824, 0.0083	1.29×10^{-6}	4.64×10^{-7}
	α_2		1.54×10^{3}	5.58×10^{2}
	α_3		1.47×10^{2}	1.06
Ratkowksy type (Eq.(6))	α_1	0.825, 0.0083	2.076×10^{-10}	1.453×10^{-10}
	α_2		3.105×10^{3}	1.099×10^{3}
	α_3		1.417×10^{-1}	1.059×10^{-1}

Table 2. Results on the parameter estimates of the secondary fitted models (Eqs. (3)- (6)) for the AA kinetics.

Equation type	Parameters	R^2_{adj}, $RMSE$	Estimated values	SE
Polynomial (Eq. (3))	β_o	0.986, 0.002	-4.011 x 10^{-4}	1.096 x 10^{-4}
	β_1		-	
	β_2		5.092 x 10^{-5}	1.480 x 10^{-5}
	β_3		3.767 x 10^{-6}	8.595 x 10^{-8}
	β_4		-	
	β_5		-2.548 x 10^{-6}	4.010 x 10^{-7}
Arrhenius type (Eq. (4))	C_o	0.928, 0.229	-6.162	0.074
	C_1		3.744 x 10^{-4}	2.425 x 10^{-5}
	C_2		-11.522	0.545
Ratkowsky type (Eq. (5))	α_1	0.939, 0.004	2.09 x 10^{-5}	1.43 x 10^{-6}
	α_2		22.73	3.472
	α_3		15.747	1.589
Ratkowksy type (Eq. (6))	α_1	0.936,0.004	7.957 x 10^{-8}	9.397 x 10^{-9}
	α_2		87.635	7.316
	a_3		15.741	1.629