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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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Quantitative modelling approaches for  
ascorbic acid degradation and  
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during ultrasound processing

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

3  
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24 **Abstract**

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

45

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

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55 **Introduction**

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

77 The nutritional quality of orange juice is primarily related to the ascorbic acid  
78 content (Zerdin et al. 2003). Ascorbic acid is thermolabile and highly sensitive to various  
79 processing conditions. The mechanism of vitamin C degradation follows aerobic and/or  
80 anaerobic pathways and depends upon several processing conditions (Tannenbaum 1976;  
81 Vieira et al. 2000). Ultrasound treatment of orange juices is reported to have a minimal  
82 effect on the ascorbic acid content during processing and results in improved stability  
83 during storage when compared to thermal treatment (Tiwari et al. 2009a). This positive  
84 effect of ultrasound is assumed to be due to the effective removal of occluded oxygen  
85 from the juice (Knorr et al. 2004), a critical parameter influencing the stability of ascorbic

86 acid (Solomon et al. 1995). Tiwari et al. (2009a) reported a maximum degradation of 5  
87 % in the ascorbic acid content of orange juice when sonicated at the highest acoustic  
88 energy density (0.81 W/mL) and treatment time (10 min). During storage at 10 °C  
89 sonicated juice was found to have a higher retention of ascorbic acid compared to  
90 thermally processed and control samples. Several studies have shown that non-thermal  
91 process technologies including high pressure, pulsed electric fields and sonication retain a  
92 higher level of ascorbic acid relative to thermally processed juices (Yeom et al. 2000;  
93 Torregrosa et al. 2006; Cheng et al. 2007; Tiwari et al. 2009a). Non-enzymatic browning  
94 (NEB) significantly influences the commercial value of citrus products, as it is the first  
95 visible quality defect to be detected during ambient temperature storage. In citrus juices,  
96 NEB may result from reactions of sugars, amino acids and ascorbic acid.  
97 Kinetic models can be used for objective, fast and economic assessments of food quality.  
98 Kinetic modeling may also be employed to predict the influence of processing on critical  
99 quality parameters. The objective of this study was to develop integrated deterministic  
100 modeling approaches of both quality indices, i.e., AA and NEB, to identify the optimal  
101 processing conditions for producing orange juice with minimal quality deterioration.  
102 Therefore, the kinetics of the quality indices of NEB and AA are described quantitatively  
103 in order to evaluate the combined effect of the extrinsic parameters of amplitude and  
104 temperature on them. The developed deterministic modeling approaches of both quality  
105 indices are integrated in order to identify the optimal conditions for producing an orange  
106 juice with minimal quality deterioration.

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108

## 109 **Materials and methods**

110

### 111 **Juice preparation**

112 Oranges (*Citrus sinensis* cv. *Valencia*) were purchased from a local fruit supplier (Reilly  
113 Wholesale Ltd., Dublin Ireland). Fresh juice was squeezed using a household table top  
114 citrus juice extractor (BRAUN GmbH, Kronberg, Germany) and filtered using a double  
115 layer cheese cloth to remove pulp. Orange juice extraction and filtration were performed

116 in a cold room maintained at  $3 \pm 1$  °C. Juice obtained was immediately frozen at -25 °C.  
117 Frozen juice samples were processed within one month of juice preparation.

118

### 119 **Ultrasound treatment**

120 A 1,500 W ultrasonic processor (VC 1500, Sonics and Materials Inc., Newtown, USA)  
121 with a 19 mm probe was used for sonication (Fig. 1). Samples were processed at a  
122 constant frequency of 20 kHz. The energy input was controlled by setting the amplitude  
123 of the sonicator probe. Extrinsic parameters of temperature (5, 10, 15, 20, 25, 30 °C),  
124 amplitude (24.4, 42.7, 61.0  $\mu$ m) and treatment time (2, 4, 6, 8, 10 min) were varied with  
125 pulse durations of 5 s on and 5 s off. Eighty mL orange juice samples were placed in a  
126 100 mL jacketed vessel through which water at a flowrate of 0.5 L/min was circulated  
127 (Fig. 1). Sonication at the desired amplitude level was started once the set temperature  
128 was reached in the jacketed beaker. The ultrasound probe was submerged to a depth of 25  
129 mm in the sample. All treatments were carried out in triplicate.

130

### 131 **Determination of non-enzymatic browning**

132 Non-enzymatic browning was measured using the method of Meydavi et al. (1977). Ten  
133 mL orange juice samples were centrifuged for 10 min; 756 *g* and  $20 \pm 0.5$  °C (Sigma 1A,  
134 AGB Scientific Ltd, Dublin, Ireland) to remove coarse particles. Five mL of ethyl alcohol  
135 (95%, Sigma-aldrich, Dublin, Ireland) was added to 5 mL of juice supernatant and  
136 centrifuged as above. The absorbance of the supernatant was obtained at 420 nm using a  
137 Unicam UV-VIS (UV2) spectrophotometer with distilled water as blank. Measurements  
138 were taken in triplicate and mean value reported. s

139

### 140 **Determination of ascorbic acid**

141 Ascorbic acid content was determined following the HPLC (Shimadzu Model no: SPD -  
142 M10AVP, Shimadzu Co., Japan) analytical procedure outlined by Lee and Coates (1999).  
143 To prepare the sample, 25 mL of the juice samples were added into 50 mL centrifuge  
144 tubes containing 5 mL of 2.5% metaphosphoric acid. Samples were centrifuged for 10  
145 min; 2000 *g* and 4 °C. Then, 5 mL of the supernatant was filtered through PTFE syringe  
146 filters (0.45 $\mu$ m, Phenomenex, U.K) and placed in an autosampler vial. Ten  $\mu$ L aliquot of



147 samples were injected onto a Shimadzu C18 (15cm × 4.6cm, pore size 5µm) coupled with  
148 HyperODS guard column. The mobile phase was 25 mM KH<sub>2</sub>PO<sub>4</sub> (adjusted to pH 3.0  
149 with phosphoric acid) at a flow rate of 1 mL/min. Eluate was monitored by UV detection  
150 at 245 nm. Chromatograms were recorded and processed with EZStart Chromatography  
151 Software V.7.2.1. Results were reported as g of ascorbic acid/L of orange juice.

152

### 153 **Overall experimental design**

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

159

### 160 **Model development**

161

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

165

$$166 \quad C(t) = C(0) + k \cdot t \quad (1)$$

$$167 \quad C(t) = C(0) \cdot \exp(k \cdot t) \quad (2)$$

168

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

175

$$176 \quad k = \beta_0 + \beta_1 \cdot T + \beta_2 \cdot T^2 + \beta_3 \cdot A + \beta_4 \cdot A^2 + \beta_5 \cdot T \cdot A \quad (3)$$

177

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

183

$$184 \quad k = C_o + C_1 \cdot A^2 + \frac{C_2}{T} \quad (4)$$

185

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

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

193

$$194 \quad \sqrt{k} = \alpha_1 \cdot (A + \alpha_2) \cdot (T + \alpha_3) \quad (5)$$

$$195 \quad \sqrt{k} = \alpha_1 \cdot (A + \alpha_2)^2 \cdot (T + \alpha_3) \quad (6)$$

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

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

200

$$201 \quad \sqrt{k} = \alpha_1 \cdot (A + \alpha_2) \cdot (1 + \exp(\alpha_3 \cdot (\alpha_4 - A))) \cdot (T + \alpha_5) \quad (7)$$

202

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

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

211

## 212 **Statistical analysis**

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

$$217 \quad RMSE = \sqrt{\frac{\sum_{i=1}^{n_t} (y_{\text{exp}}(t_i) - y(t_i, p_{ls}))^2}{n_t - n_p}} \quad (8)$$

218

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

221

$$222 \quad R^2_{adj} = 1 - \left( \frac{n_t - 1}{n_t - n_p} \right) \cdot \frac{SSE}{SSTO} \quad (9)$$

223

224 Herein,  $SSTO$  is the total sum of squared errors  $\sum (y_i - \bar{y})^2$  and  $SSE$  the sum of squared

225 errors  $\sum (y_{\text{exp}}(t_i) - y(t_i, p_{ls}))$ .

226

## 227 **Software Programs**

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

233

## 234 **Results & Discussion**

235

### 236 **Non-enzymatic browning (NEB)**

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

251 The lower P-values (so the more 'significant' the results) were obtained for the  
252 coefficient of  $\beta_4$  ( $P=0$ ) (quadratic effect of Amplitude) and  $\beta_2$  ( $P=1.08 \times 10^{-26}$ ) (linear  
253 effect of temperature) indicating that for the same temperature levels the NEB rate  
254 constants were highly dependent on the amplitude levels followed by the temperature  
255 levels. Interactive effects gave higher P values ( $1.36 \times 10^{-6}$ ). The NEB rate significantly  
256 increased with processing temperature while at intermediate ultrasound amplitudes  
257 appeared to have lower values indicating an antagonistic effect of amplitude on the rate  
258 of NEB. More specifically, at amplitude levels of the range of 42.7  $\mu\text{m}$ , the NEB rate  
259 appeared to be lower than at higher or lower amplitude levels for the same temperatures.  
260 The observed monotonic increase of the NEB rate with respect to temperature has also  
261 been reported for the browning kinetics of apple juice and apple cider (Ugarte-Romero et  
262 al. 2006; Vaikousi et al. 2008). Nonenzymatic browning may result from the  
263 condensation of a carbonyl group with amino acids, reactions of sugars and ascorbic acid  
264 in the absence of free amino acids (caramelization). The obtained increase of the

265 browning rate at high amplitudes can be attributed to the decrease of sugar content (Yuan  
266 et al. 2009).

267

### 268 **Ascorbic acid degradation (AA)**

269

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

275 The largest AA reduction was observed at the highest amplitude (61.0  $\mu\text{m}$ ) and  
276 processing temperature (30  $^{\circ}\text{C}$ ), . However this reduction was less than 15% loss of the  
277 initial ascorbic acid content of the unprocessed juice. The ascorbic acid rate constant with  
278 respect to the amplitude and the temperature was described more accurately by  
279 employing the polynomial model (Eq. (3)) (Table 2).

280 The lower P-values were obtained for the coefficient of  $\beta_3$  ( $P=0$ ) (linear effect of  
281 amplitude) followed by  $\beta_5$  ( $P=6.16 \times 10^{-8}$ ) (interactive effect of amplitude and  
282 temperature). Fig. 3 illustrates that increase of temperature and increase of amplitude  
283 resulted in higher ascorbic acid loss. This indicates a synergistic effect of temperature for  
284 different amplitudes and temperatures on the AA rate constant.

285 Several mechanisms can act concurrently when ultrasound is applied in liquid systems,  
286 i.e., thermal effects produced by bubble implosion, mechanical stresses produced  
287 microstreaming and implosion shock waves, and free radical production. Nevertheless,  
288 radical productions have been considered the most probable mechanism (Portenlanger  
289 and Heusinger 1992; Vercet et al. 2001). The degradation of ascorbic acid divides into  
290 two sections corresponding to aerobic and anaerobic degradation (Nagy 1980;  
291 Eisonperchonok and Downes 1982; Robertson and Samaniego 1986; Kennedy et al.  
292 1992; Ariaahu et al. 1997; Blasco et al. 2004). Sonication results in a reduction of  
293 dissolved oxygen, a critical parameter influencing the stability of ascorbic acid (Solomon  
294 et al. 1995). Hydroxyl radical formation is found to increase with degassing. Sonication  
295 cavities can be filled with water vapour and gases dissolved in the juice such as  $\text{O}_2$  and

296 N<sub>2</sub> (Korn et al. 2002). The interactions between free radicals and ascorbic acid may occur  
297 at the gas–liquid interfaces. In summary ascorbic acid degradation may follow one or  
298 both of the following pathways:

299 Ascorbic acid → thermolysis (inside bubbles) and triggering of Maillard reaction

300 Ascorbic acid → reaction with OH<sup>-</sup> → HC–OH and production of oxidative products on  
301 the surface of bubbles

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

307

### 308 **Contour design**

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

322

323

### 324 **Conclusion and future work**

325 The modeling approaches developed in this study exploit data in order to identify the  
326 optimal processing regions for eliminating quality deterioration of orange juice during

327 ultrasound processing which is of high importance in food industry. The non-monotonic  
328 behaviour of NEB has been described more accurately by the use of a polynomial model.  
329 The rate constants of AA were described by a similar type of model having a monotonic  
330 behaviour. A synergistic effect of temperature for different amplitudes on the rate  
331 constant of both NEB and AA was observed, while an antagonistic effect of amplitude on  
332 the rate of NEB was evident. Ultrasound was found to have more drastic effect on NEB  
333 than AA degradation of orange juice.

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

345

346

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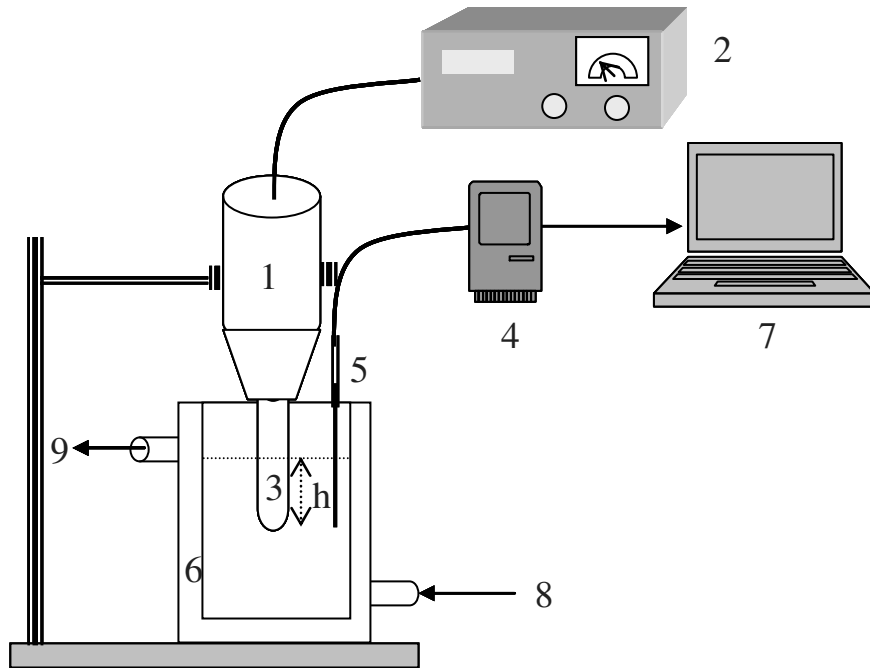
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470 Figure 1. Experimental setup (1) ultrasound transducer; (2) ultrasonic generator; (3)  
471 ultrasound probe (19 mm); (4) data logger; (5) temperature probe; (6) jacketed beaker; (7)  
472 computer; (8) water inlet; (9) water outlet; (h) depth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

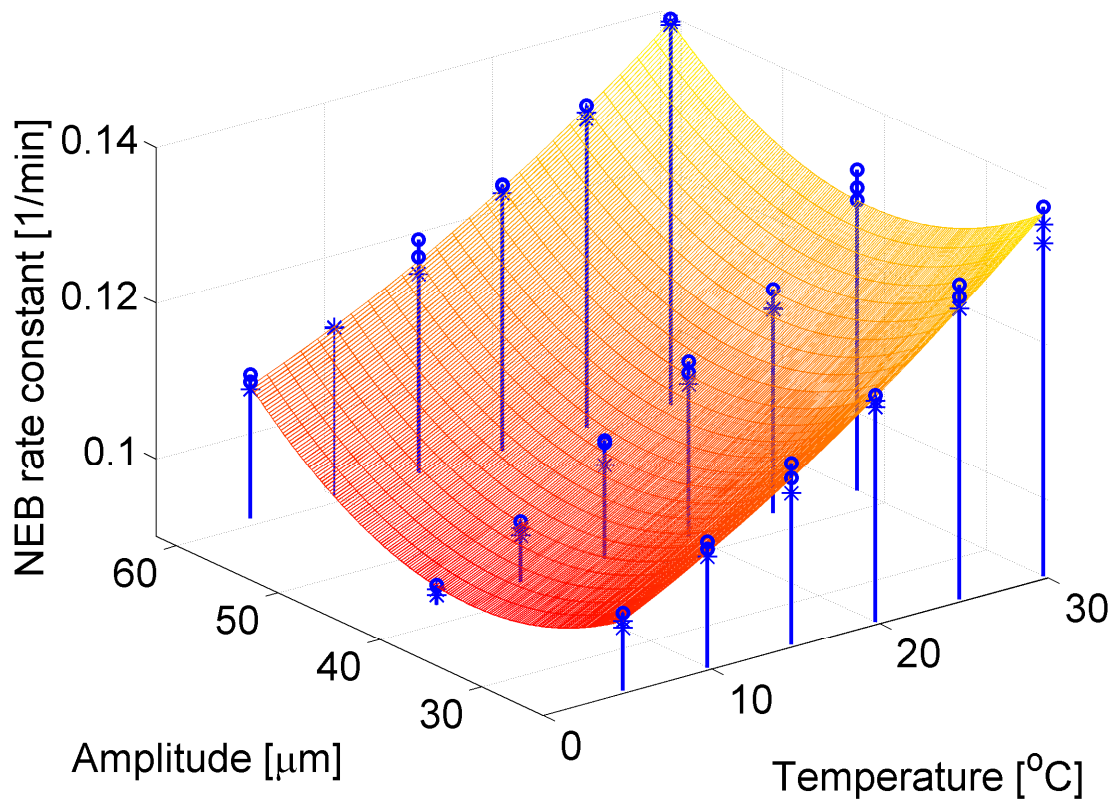
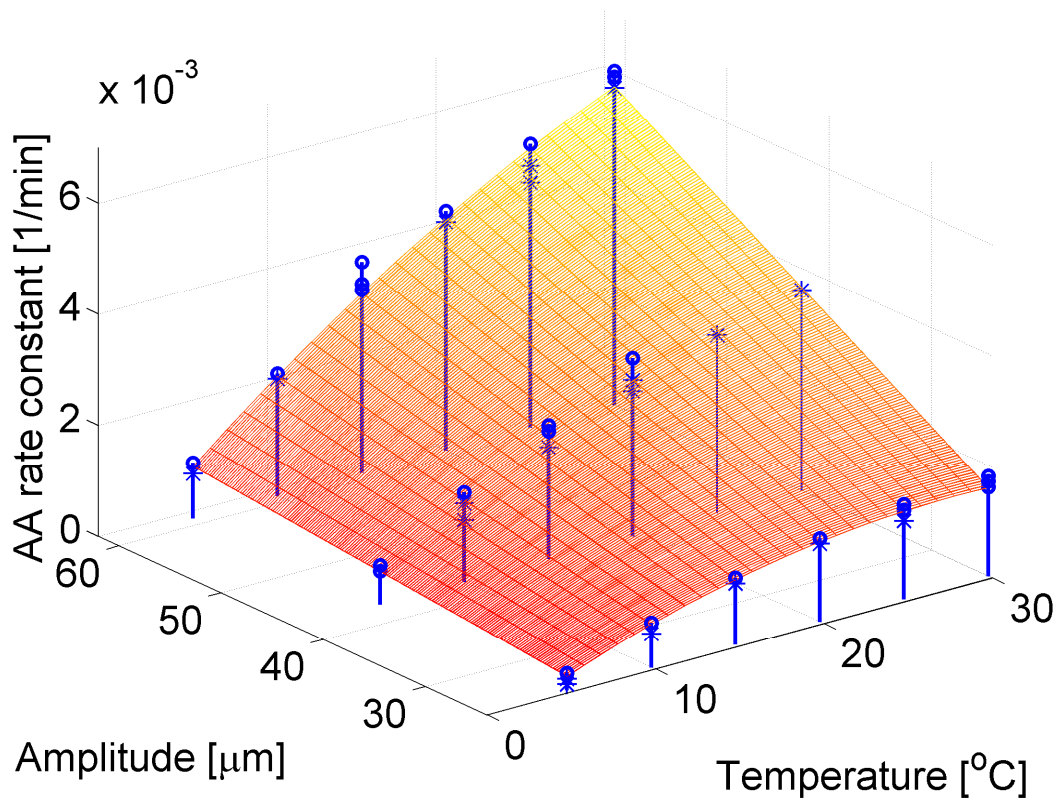


Figure 2. Modelling the non enzymatic browning rate constant,  $k$  (Eq. (3), Table 1). (o): experimental data points above the surface, (\*): experimental data points under the surface.



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506 Figure 3. Modelling the ascorbic acid rate constant,  $k$  (Eq. (3), Table 2). (o):

507 experimental data points above the surface, (\*): experimental data points under the

508 surface.

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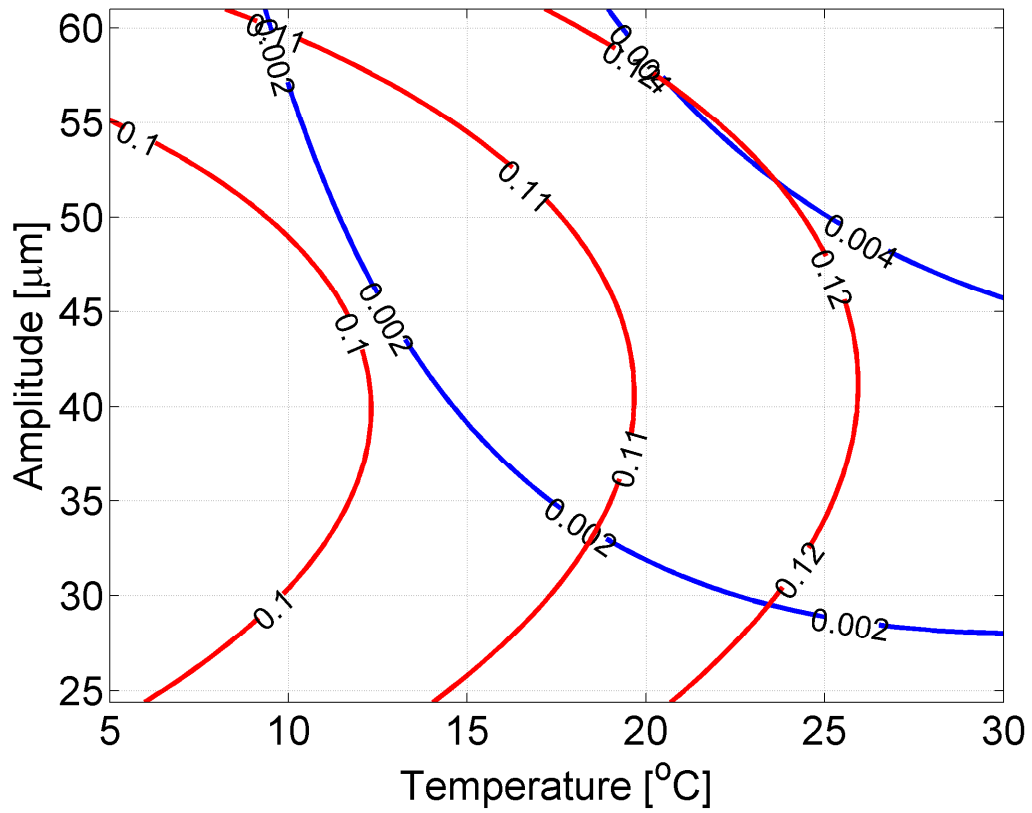


Figure 4. Non enzymatic browning and ascorbic acid iso-rate contour plots for  $k = 0.1$ ,  $0.11$ ,  $0.12$  [1/min] and  $k = 0.004$ ,  $0.004$  [1/min], respectively.

546 Table 1. Results on the parameter estimates of the secondary fitted models (Eqs. (3)- (6))  
 547 for the NEB kinetics.  
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Equation type	Parameters	$R^2_{adj}, RMSE$	Estimated	SE
			values	
Polynomial (Eq. (3))	$\beta_0$	0.998, 0.0015	0.135	0.003
	$\beta_1$		-2.500	$1.127 \times 10^{-4}$
	$B_2$		1.000	$1.331 \times 10^{-4}$
	$\beta_3$		-5.964	$1.571 \times 10^{-6}$
	$\beta_4$		3.193	$1.270 \times 10^{-6}$
	$\beta_5$		1.773	$3.214 \times 10^{-6}$
Arrhenius type (Eq. (4))	$C_0$	0.6187, 0.072	-2.078	0.023
	$C_1$		$1.927 \times 10^{-5}$	$7.649 \times 10^{-6}$
	$C_2$		-1.553	0.172
Ratkowsky type (Eq. (5))	$\alpha_1$	0.824, 0.0083	$1.29 \times 10^{-6}$	$4.64 \times 10^{-7}$
	$\alpha_2$		$1.54 \times 10^3$	$5.58 \times 10^2$
	$\alpha_3$		$1.47 \times 10^2$	1.06
Ratkowsy type (Eq.(6))	$\alpha_1$	0.825, 0.0083	$2.076 \times 10^{-10}$	$1.453 \times 10^{-10}$
	$\alpha_2$		$3.105 \times 10^3$	$1.099 \times 10^3$
	$\alpha_3$		$1.417 \times 10^{-1}$	$1.059 \times 10^{-1}$



549 Table 2. Results on the parameter estimates of the secondary fitted models (Eqs. (3)- (6))  
 550 for the AA kinetics.  
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Equation type	Parameters	$R^2_{adj}, RMSE$		
		Estimated values	SE	
Polynomial (Eq. (3))	$\beta_0$	0.986, 0.002	$-4.011 \times 10^{-4}$	$1.096 \times 10^{-4}$
	$\beta_1$		-	
	$\beta_2$		$5.092 \times 10^{-5}$	$1.480 \times 10^{-5}$
	$\beta_3$		$3.767 \times 10^{-6}$	$8.595 \times 10^{-8}$
	$\beta_4$		-	
	$\beta_5$		$-2.548 \times 10^{-6}$	$4.010 \times 10^{-7}$
Arrhenius type (Eq. (4))	$C_0$	0.928, 0.229	-6.162	0.074
	$C_1$		$3.744 \times 10^{-4}$	$2.425 \times 10^{-5}$
	$C_2$		-11.522	0.545
Ratkowsky type (Eq. (5))	$\alpha_1$	0.939, 0.004	$2.09 \times 10^{-5}$	$1.43 \times 10^{-6}$
	$\alpha_2$		22.73	3.472
	$\alpha_3$		15.747	1.589
Ratkowsky type (Eq. (6))	$\alpha_1$	0.936, 0.004	$7.957 \times 10^{-8}$	$9.397 \times 10^{-9}$
	$\alpha_2$		87.635	7.316
	$\alpha_3$		15.741	1.629

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