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Abstract

Degradation of the mycotoxin patulin between 25-85 ^{o}C without and with added ascorbic acid was studied, and the usefulness of linear and non-linear models for predicting reaction rates was compared. In agreement with previous reports, ascorbic acid significantly increased ($p \leq 0.05$) the rate of patulin degradation at all temperatures studied. The data for patulin degradation in the absence of ascorbic acid were adequately modeled using a zero-order linear kinetic model. However, the predictive abilities of zero and higher order linear models were not adequate to describe the more complex reactions that likely occurred when ascorbic acid was added. In contrast, the non-linear Weibull model adequately described the patulin-ascorbic acid reaction throughout the temperature range studied. Zero-order rate constants and Weibull scale values for each of the respective reactions followed the Arrhenius law. Activation energies of 58.7 ± 3.9 and 29.6 ± 1.9 kJ mol^{-1} for the reaction without and with ascorbic acid, respectively, confirmed decreased patulin stability in the presence of ascorbic acid and suggested that the mechanisms for the two degradation reactions were different.

Keywords: Patulin, Ascorbic acid, Kinetics, Thermal degradation, Weibull model.

Practical application

Ascorbic acid is known to increase the rate of degradation of patulin in apple juice. Because this vitamin is Generally Recognized As Safe (GRAS), inexpensive, recognized by consumers as a beneficial nutrient, and does not negatively affect the sensory properties of apple juice, it may find application for reducing levels of this mycotoxin.

Kinetic studies on the reaction of ascorbic acid and patulin are useful for providing information about the reaction mechanism and for process optimization studies to determine thermal processing schemes and post processing storage conditions that can be used to minimize patulin levels in apple juice.

Introduction

Patulin is a mycotoxin produced by several mold genera including *Penicillium, Aspergillus* and *Byssochlamys*. *P.expansum,*a common cause of post-harvest decay of apples, is the most common source of patulin contamination in apples and processed apple products (Paster and others 1995).Animal and cell studies have demonstrated a variety of acute and chronic effects exerted by patulin including neurotoxicity, immunotoxicity, carcinogenicity, teratogenicity, and mutagenicity (Alves and others 2000; Escoula and others 1988; Geiber and Conn 1945; Mayer and Legator 1969; Paucod 1990; Sharma 1993; Taniwaki 1992). Although only indirect evidence suggests that patulin is harmful to humans, data from animal toxicological studies and human consumption rates have been used by international food regulatory agencies to establish maximum allowable levels. In the U.S., the Food and Drug Association (FDA) has established an action level for patulin in single strength apple juice of 50μg/kg (50ppb) (FDA 2001) and requires that patulin control measures be included in FDA mandated Juice Hazards Analysis Critical Control Point (HACCP) plans (FDA 2004).In other countries, maximum allowable levels as low as 25 μg/kg have been mandated (FAO 2002).

Post harvest methods for minimizing patulin levels in apple juice include the use of fungicides, bio control agents, and controlled atmospheric storage to inhibit mold growth (Morales and others 2010)FDA recommendations are to purchase only sound, tree picked apples, and to use culling, trimming and washing methods to remove decayed apple tissue. However, these methods may not be reliable since patulin may diffuse into apple tissues where decay is not apparent during visual inspection(Beretta 2010).

A comprehensive review of factors affecting patulin stability in apple products was conducted by (Moake and others 2005). Although degradation is more rapid at higher pH values (Drusch and others 2007), patulin is relatively stable during heat processing in acidic apple juice. Addition of sulfite, sulfur dioxide, cysteine, and glutathione have been shown to reduce patulin levels during processing and storage (Ciegler and others 1976; Fliege and Metzler 2000a, 2000b). Of particular interest are studies that have demonstrated accelerated degradation of patulin in the presence of ascorbic acid (Alves and others 2000; Aytac and Acar 1994; Canas and Aranda 1996; Drusch and others 2007; Fremy and others 1995). Ascorbic acid is Generally Recognized As Safe (GRAS) when used in accordance with Good Manufacturing Practices (21CFR 182.3013,2000), is relatively inexpensive, and is widely recognized by consumers as a beneficial nutrient (vitamin C).There is some evidence that patulin reductions are accompanied by a decrease in genotoxicity (Alves and others 2000). Thus, the use of ascorbic acid shows promise as a method for controlling the hazards presented by this mycotoxin. Although the effectiveness of ascorbic acid has been shown repeatedly, there have been no controlled kinetic studies conducted that could provide insight on the reaction mechanism.

The most common approach for modeling microbial lethality or chemical degradation in foods has been to use zero, first, or second-order kinetic models (Villota and Hawkes 2007). However for complex reactions, the rate at any time, determined by applying linear regression, may be over- or under-estimated depending on how well the model fits the experimental data. In order to overcome these limitations, the more flexible non-linear Weibull model has been used to incorporate parameters for both curve shape and rate (Corradini and Peleg 2004). The Weibull model has found useful application for modeling inactivation of microorganisms (Chen and Hoover 2004; Huang 2009; Keklik and others 2012; Van Boekel 2002) as well as degradation of nutrients, pigments and enzymes during food processing and storage (Corradini and Peleg 2004; Mafart and others 2002; Manso and others 2001; Odriozola-Serrano and others 2009; Oms-Oliu and others 2009; Tiwari and others 2009; Zheng and Lu 2011).

Accurate prediction of degradation reaction rates in foods may require different mathematical models depending on the compound studied. Odriozola-Serrano and others (2009) reported that a Weibull kinetic model most accurately estimated changes in anthocyanins and antioxidant capacity of fresh-cut strawberries during storage, although a first-order model was adequate for predicting loss of vitamin C. Zheng and Lu (2011) determined that the first-order kinetic model was adequate for predicting reductions of ascorbic acid and total phenols during pasteurization of pineapple juice. However, in the same juice samples, the Weibull model more accurately predicted changes in radical scavenging activity.

An understanding of the kinetics of the degradation of patulin in the presence of ascorbic acid is necessary to further explore the usefulness of this approach for

controlling mycotoxin levels in apple juice. The objective of this study is therefore to use statistical methods to compare the ability of linear models and the non-linear Weibull model for predicting the degradation of patulin without or with ascorbic acid added.

Materials and Methods

Chemicals

Patulin (≥ 98% pure, crystalline), ascorbic acid, and malic acid were obtained from Sigma Aldrich, (St. Louis, Mo., U.S.A). Acetonitrile (HPLC grade), water (HPLC grade), and formic acid (reagent grade) used for High Performance Liquid Chromatography (HPLC) analysis were obtained from Fisher Scientific (Fair Lawn, N.J., U.S.A).

Reaction medium

Experiments were conducted using an apple juice model system consisting of 0.5% malic acid adjusted to pH 3.75. Values for malic acid concentration and pH were based on typical levels reported in apple juice (Mattick and Moyer 1983).The concentration of ascorbic acid chosen is based on the range of recommend daily intake values for single serving of juice. Addition of NaOH (1M) solution was used to vary pH and measurements were performed using an Accumet AR25 dual channel pH/ion meter (Fisher Scientific, Fair Lawn, N.J., U.S.A).

Sample preparation and treatment

Stock solutions of patulin were prepared according to AOAC procedures (MacDonald and others 2000). Five (5.00) mg of patulin was dissolved in 5 mL of ethyl

acetate in a 25-ml volumetric flask, brought to volume with ethyl acetate, and stored at -20^{o}C until used. To prepare reaction solutions, the stock solution was warmed to room temperature, 1.0 mL was transferred to a test tube, and the contents were dried under N_2 gas. Immediately after drying, 4.0 ml of distilled water acidified to pH 4.0 with acetic acid was added and the solution was vigorously vortexed for 30 sec. The aqueous solution containing approximately 50,000 ppb patulin was kept at 4^{o}C until use. Before each experiment, the actual concentration of patulin was determined by HPLC.

Prior to each experiment, 0.5% malic acid buffer was added to a 250ml Erlenmeyer flask, which was then immersed in a temperature controlled recirculating water bath (NESLAB RTE-17, Thermo Scientific, Waltham, Mass., U.S.A) for 30 min to allow for temperature equilibration. The water bath temperature was monitored using a digital thermocouple and was maintained to a precision of ± 0.5^{o}C. The thermocouple was checked against a certified reference thermometer that was calibrated annually by an NIST laboratory. The level of water was maintained at least 1.5 inches above the reaction solution level in the flask to ensure uniform temperature distribution. The flask was then removed from the water bath and appropriate amounts of patulin stock solution and ascorbic acid were added to achieve a final volume of 50 ml and respective concentrations of 6.40μM (1000ppb) and 1.25 mM (221ppm). The mixture was mechanically stirred for 20 sec with a stir bar after which the flask was immediately returned to the water bath. The change in volume caused by addition of reactants did not change the reaction solution volume by more than 2%. At each reaction time interval, 1.0 mL of sample was taken from the reaction vessel for HPLC patulin analysis and the flask was immediately returned to the water bath. The range of temperatures (25-85^{o}C) for

experiments was chosen to simulate conditions that may occur during thermal processing and post processing storage of shelf stable juice. Each reaction was followed until patulin was below the detection limit or when no further significant changes in concentration occurred.

Patulin analysis

Patulin was quantified using a Waters HPLC system (pump model: 600; Autosampler: 71P; photodiode array (PDA) detector: 2998, Waters Inc., Milford, Mass., U.S.A.) and a C-18 reverse phase/cation exchange column (Primesep-D, 4.6 mm × 150 mm, particle size 5 μm, SIELC Inc., Prospects Heights, Ill., U.S.A.). A two-solvent gradient elution system was used. Solvent A consisted of water containing 0.1% formic acid adjusted to pH 1.85 with HCL. Solvent B was acetonitrile. Gradient conditions (v/v) were as follows: 98% A: 2% B at 0 min, 95% A: 5% B at 3 min; 90% A: 10% B at 5 min, 85% A: 15% B at 7 min. The flow rate was maintained at 1 mL/min. At each reaction time interval, 20 μL was injected directly onto the column without prior extraction or clean up. The identity of patulin was confirmed by comparing retention times and maximum absorbance wavelength (4.4 min and 276.0 nm, respectively). Standard curves for patulin were prepared by plotting the concentration of prepared aqueous solutions vs. peak area at A_{max}. The detection limit for patulin was 0.165μM (25ppb).

Kinetic models

Patulin degradation was modeled using the following zero-order (Eq. 1), first-order (Eq. 2), and second-order (Eq. 3), equations:

$$C_t = C_0 - (k_0 \cdot t) \quad \text{Eq. (1)}$$

$$C_t = C_0 \cdot \exp(-k_1 \cdot t) \quad \text{Eq. (2)}$$

$$1/C_t = 1/C_0 + (k_2 \cdot t) \quad \text{Eq. (3)}$$

Where C_t and C_0 are patulin concentrations, at time t and zero, respectively and t is the reaction time. k_0, k_1, and k_2 are zero, first, and second-order rate constants, respectively.

The data were also used to fit the Weibull model from Corradini and Peleg (2004):

$$C_t/C_0 = \exp(-b \cdot t^n) \quad \text{Eq. (4)}$$

Where b = the Weibull scale parameter (min^{-n}) and n = the shape parameter. The scale parameter (b) is analogous to a rate constant and as such is usually dependent on reaction temperature. The shape parameter (n) mathematically describes the shape of the degradation curve. When n>1, the degradation rate increases with time and when n<1, the rate decreases with time. When n=1, the equation is equivalent to a simple first-order kinetic model. The value for the shape parameter remains constant with increasing temperature if the reaction mechanism is unaffected by temperature.

Temperature dependency

The Arrhenius equation was used to compare the temperature dependency of patulin degradation according to the equation:

$$\ln(k \text{ or } b) = -E_A/RT + \ln A \quad \text{Eq. (5)}$$

where k = the rate constant for the reaction, b = the Weibull scale parameter, Ea = activation energy ($J\ mol^{-1}$), R = the gas constant (8.314 $J\ mol^{-1}K^{-1}$), T = Temperature (^OK), and A = the Arrhenius pre exponential factor.

Statistical analysis

Experiments for each treatment and temperature were conducted in triplicate. SAS statistical software (Release 9.1.3 SAS institute Inc, Carry, NC, U.S.A.) and

GraphPad Prism (Version 5.0d GraphPad Software Inc.) were used for 2-way ANOVA determination of the significance of reaction parameters, and to determine apparent zero, first, and second-order rate constants, Weibull scale (b) and shape (n) factors, and the goodness of fit of the experimental data to each model. Regression coefficients (R^2) described the percentage of variability within the data explained by the model. The standard deviation for residuals (Sy.x) was used to measure how close the data were to the fitted regression line. Residual plots as a function of reaction time were presented as graphical inserts within each figure. For each model, Wald-Wolfowitz tests, known as the Runs test for randomness, were used to test the null hypothesis that there is no systematic deviation from the curve generated by the model. A p-value less than or equal to 0.05 indicates a non-random distribution of residuals and thus a significant deviation from the model.

Results and Discussion

Data for the degradation of patulin without and with ascorbic acid added were followed for up to 148 hours. Decreases in patulin concentration were significantly ($p<0.001$) affected by reaction temperature and the presence of added ascorbic acid. These results are in agreement with earlier studies on factors affecting patulin degradation in model systems and processed juice ((Alves and others 2000; Aytac and Acar 1994; Canas and Aranda 1996; Drusch and others 2007; Fremy and others 1995) .

Patulin degradation without added ascorbic acid

Data for the degradation of patulin (C_0 = 6.40μM) at 25, 35, 45, 55, 65, and 85^{o}C in samples with no added ascorbic acid and fitted with a zero-order kinetic model are

presented in **Figure 1**. Inserted in this figure, and in subsequent figures, is a residual plot for the reaction at 45^{o}C. Residual plots at this temperature were representative of trends that occurred at all temperatures studied for each of the models evaluated for reactions without or with ascorbic acid. In **Table 1,** rate constants and statistical results to evaluate the fit of the zero-order model to the experimental data are shown.

The apparent random distribution of residuals with reaction time **(Figure 1)** confirms goodness of fit for the zero-order model. The fit of the zero-order model to data obtained at all reaction temperatures was high as evidenced by R^2 values of at least 0.959 and consistently low Sy.x values at each temperature **(Table 1)**. Generated p-values by runs tests were well above 0.05 for all temperatures, indicating that deviations from the zero order kinetic model were not statistically significant. When fits of first and second-order models to the experimental data were compared to the zero-order model, no meaningful increases in R^2 values or decreases in p values occurred, thus indicating that no statistically significant deviations from each of the linear models occurred (data not shown). Therefore, the zero-order rate model, within these experimental conditions, can be used to predict the extent to which patulin degradation occurs when no ascorbic acid is added.

Patulin degradation with added ascorbic acid

In **Figure 2a, b, and c**, respective fits of a) zero, b) first, and c) second-order kinetic models are compared for the degradation of patulin with added ascorbic acid at 25, 35, 45, 55, 65, and 85^{o}C respectively. In contrast to the good fit of the zero-order kinetic model to data obtained for patulin degradation when no ascorbic acid is added **(Figure 1)**, it is visually apparent from **Figure 2a** that this model is not satisfactory. The residual plot

insert presents a non-random distribution and shows a systematic underestimation of patulin degradation during the initial part of the reaction and overestimation at longer times indicating the poor fit of the model. As expected, zero-order rate constants (k_0) for patulin degradation when ascorbic acid is added (**Table 2)** were consistently higher compared to k_0 values obtained when no ascorbic acid was added **(Table 1)**. However, the fit of the zero-order model was poor as evidenced by R^2 values between 0.663 and 0.858 and comparatively higher Sy.x values at each temperature. Runs test yielded p values were consistently less than 0.05, thus indicating systematic deviation from the zero-order model occurred at all temperatures except 85^{o}C.

When the fit of first and second-order models to the data were compared (**Figure 2b and 2c)**, there was little or no improvement compared to the zero-order model. Plots of the residuals at each reaction time again showed that predicted values underestimated and then overestimated experimental values as reaction time increased. Although changes in k_1 and k_2 values in **Table 2** confirmed the positive effect of temperature on reaction rate, the statistical data show only moderate improvement in the predicative ability of the first and second-order kinetic model compared to the zero-order model. Respective R^2 value ranges among the reaction temperatures increased from between 0.824 and 0.920 for the first-order model to 0.871 to 0.971 for the second-order model. Standard deviation values for the residuals (Sy.x) for both linear models tended to increase with increasing temperatures. Systematic deviation of the first-order model to the experimental data was significant ($p \leq 0.05$) for all reaction temperatures and deviation of the second-order model was significant at all temperatures except 35 and 85^{o}C. These results indicate that simple

linear kinetic models are not adequate for predicting the extent to which patulin degradation occurs between 25 and 85 ^{o}C when added ascorbic acid is present.

In summary, thermal degradation of patulin in the absence of ascorbic acid between 25 and 85 ^{o}C can be adequately described by a simple zero order kinetic model. However, it appears that addition of ascorbic acid increases the complexity of the degradation reaction and leads to inaccurate prediction of reaction rates when simple linear kinetic models are used.

Weibull Model.

Figure 3a illustrates the fit of the Weibull model to the data for degradation of patulin with added ascorbic acid within the same temperature range. In contrast to results for the linear models (**Figure 2)**, it is visually apparent from the residual plot insert that the Weibull residuals are more randomly distributed. Values for the Weibull scale parameter (b) in **Table 3** increased as the temperature was raised from 25 to 85^{o}C, which was expected given that the scale parameter is analogous to rate constant (k) used in linear kinetic models. Shape parameter (n) values did not significantly ($p>0.05$) differ between 25 and 85^{o}C ($\bar{n}$ = 0.453 $\pm$ 0.033). All R^2 values were very high (0.987 – 0.999) with no apparent trend with respect to temperature. Sy.x values also changed little with temperature. Runs test p-values were well above 0.05 for each temperature indicating consistent goodness of fit of the Weibull model to the experimental data. These results show that the predictive ability of the non-linear Weibull model is superior to linear models for the degradation of patulin when ascorbic acid is added under the conditions present in this study.

Because the shape parameter (n) was independent of temperature, it can be assumed that the reaction mechanism remains the same through the entire temperature range studied (Corradini & Peleg, 2004). Thus, **Equation 4** can be simplified by replacing the variable term n with the average value obtained within the temperature range studied to yield a simplified Weibull equation as follows:

$$C/Co = \exp(-b \cdot t^{0.453}) \quad \text{(Eq. 6)}$$

The plot for the fit of the simplified Weibull model to the data and the residual plot insert in **Figure 3b** appears very similar to the full Weibull plot in **Figure 3a**. Analysis of the statistical data for simplified Weibull in **Table 4** showed again that b values increase at higher temperatures, all R^2 values remained very high (0.985 – 0.999), and Sy.x values were low with no apparent upward or downward trend with increasing temperature. There were no significant deviations of the fit of the simplified Weibull to the experimental data as evidenced by runs tests p-values well above 0.05. Thus, the superior predictive ability of the Weibull model in **Equation 4** is not negatively affected by simplification to **Equation 6**.

Reaction temperature dependency.

A comparison of temperature dependency for the degradation of patulin without and with ascorbic acid added is shown by in the Arrhenius plot in **Figure 4.** Based on the previous results in this study, zero-order rate constants were used to quantify patulin degradation rates in the absence of added ascorbic acid while simplified Weibull scale parameters (b) were used for the degradation of the patulin when ascorbic acid was added. The relationship between $\ln(k_0)$ or ln(b) versus the inverse of the absolute reaction temperature (1/T) were highly linear (R^2=0.985 and 0.987), respectively, indicating an

Arrhenius type temperature dependency for both reactions. Calculated activation energies for patulin degradation without and with added ascorbic acid were 58.7 and 29.6 $kJmol^{-1}$, respectively. Both values are in the range reported for other food chemical reactions (Villota and Hawkes 2007).The lower activation energy observed for the degradation of patulin in the presence of ascorbic acid is consistent with the accelerating effect of ascorbic acid reported in this and other studies, and suggests that the two reactions occur via different mechanisms.

From the kinetic data presented in this study, it can be hypothesized that, in the absence of ascorbic acid, patulin degradation occurs by a simple single-step reaction that is well predicted by the zero-order kinetic model. However, addition of ascorbic acid increases the complexity of the degradation reaction as evidenced by the poor fit of zero, first, and second-order linear models **(Figure 2, Table 2).**

Studies by Fliege and Metzier (2000a, 2000b) and Cielger and others (1976) provided evidence that the electrophilic properties of patulin make it a suitable target for nucleophilic attack via Michael addition with the resulting formation of stable adducts. Although these studies were limited to the effects of sulfhydryl compounds, it can be hypothesized that ascorbic acid, acting as a nucleophile, accelerates patulin degradation by a similar complex mechanism. Alternatively, oxidative degradation of ascorbic acid may form reactive free radicals that attack the lactone structure of patulin (Brackett and Marth 1979; Drusch and others 2007). It is possible that both degradation mechanisms occur, the predominance of either one depending on the relative amount of ascorbic acid and oxygen present in solution as the reaction proceeds. Further elucidation of the mechanistic pathway for this reaction can be achieved by LC/MS identification of

intermediate and end products. Structural identification and conformation may then be achieved through NMR spectroscopy and isotopic carbon module labeling techniques. Isolation and purification of intermediate and final products should be followed by cellular and animal toxicity studies to determine if the disappearance of patulin when ascorbic acid is added is accompanied by detoxification.

Conclusions

The results of this study are in agreement with previous studies in model and juice systems showing that ascorbic acid increases the rate of patulin degradation. Although the zero-order kinetic model was adequate for describing the degradation of patulin in the absence of ascorbic acid, zero-order and higher linear models did not adequately describe the more complex reaction that occurs when ascorbic acid is present. In contrast, the Weibull model, and its simplified form, more accurately predicted patulin degradation when ascorbic acid was added, and therefore, may be useful for determining intrinsic and extrinsic factors affecting the destruction of patulin in the presence of ascorbic acid and to validate predictive models applicable to apple juice. The information gained could then be used to determine thermal processing schemes and post processing storage conditions to minimize patulin levels in apple juice containing added ascorbic acid. In this study, the reaction of patulin with ascorbic acid was studied in a model system. Therefore, further research is needed to validate the usefulness of the predictive models in apple juice within typical concentration ranges of chemical juice constituents and pH levels. The information gained could then be used to determine thermal processing schemes and

post processing storage conditions to minimize patulin levels in apple juice containing added ascorbic acid.

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Table 1. Fit of the zero-order model for the degradation of patulin (C_0 = 6.40 μM) without added ascorbic acid at 25, 35, 45, 55, 65, and 85°C.

Kinetic model		Kinetic constant	Statistical data			
Zero order	**T (°C)**	**k_0 (SD)** [1] **($M \cdot hr^{-1}$)**	**R^2**	**SD residuals (Sy.x)**	**Runs test (p-value)**	**Dev. from model**[2]
	25	0.0003 (0.00002)	0.959	0.0029	0.6457	NS
	35	0.0005 (0.00002)	0.966	0.0044	0.4126	NS
	45	0.0009 (0.00003)	0.987	0.0047	0.2867	NS
	55	0.0018 (0.00007)	0.989	0.0092	0.0775	NS
	65	0.0030 (0.00120)	0.967	0.0216	0.1212	NS
	85	0.0157 (0.00084)	0.984	0.0341	0.0714	NS

[1] Each value represents the average of three determinations. Standard deviations (SD) are shown in parentheses.
[2] S and NS denote that deviation of the fit of the models to the data is significant or not significant, respectively (α = 0.05).

Table 2. Comparison of the fit of zero, first, and second-order models to experimental data for the degradation of patulin (C_0 = 6.40 μM) with added ascorbic acid (C_0 = 1.25 mM) at 25, 35, 45, 55, 65, or 85 ^oC.

Kinetic Model		Kinetic constants	Statistical data			
Zero-order	**T (^oC)**	**k_0 (SD)** [1] **($M \cdot hr^{-1}$)**	**R^2**	**SD residuals (Sy.x)**	**Runs test (p-value)**	**Dev. from model** [2]
	25	0.0024 (0.0003)	0.772	0.0600	0.0009	S
	35	0.0031 (0.0003)	0.858	0.0567	0.0022	S
	45	0.0039 (0.0005)	0.780	0.0889	0.0009	S
	55	0.0042 (0.0006)	0.736	0.1102	0.0009	S
	65	0.0510 (0.0007)	0.725	0.1105	0.0002	S
	85	0.0138 (0.0037)	0.633	0.1772	0.0833	NS
First-order	**T (^oC)**	**k_1 (SD) (hr^{-1})**	**R^2**	**SD residuals (Sy.x)**	**Runs test (p-value)**	**Dev. from model**
	25	0.0031 (0.0004)	0.824	0.0676	0.0009	S
	35	0.0046 (0.0003)	0.920	0.0602	0.0022	S
	45	0.0069 (0.0006)	0.898	0.1000	0.0014	S
	55	0.0083 (0.0008)	0.879	0.1340	0.0014	S
	65	0.0116 (0.0009)	0.886	0.1457	0.0002	S
	85	0.0453 (0.0048)	0.918	0.2274	0.0476	S
Second-order	**T (^oC)**	**k_2 (SD) ($M^{-1} \cdot hr^{-1}$)**	**R^2**	**SD residuals (Sy.x)**	**Runs test (p-value)**	**Dev. from model**
	25	0.0042 (0.0004)	0.871	0.0757	0.0009	S
	35	0.0069 (0.0003)	0.961	0.062	0.1421	NS
	45	0.0130 (0.0005)	0.974	0.092	0.0014	S
	55	0.0179 (0.0008)	0.971	0.1345	0.0357	S
	65	0.0296 (0.0011)	0.974	0.1677	0.0011	S
	85	0.2087 (0.0160)	0.956	0.7526	0.1667	NS

[1] Each value represents the average of three determinations. Standard deviations (SD) are shown in parentheses.

[2] S and NS denote that deviation of the fit of the models to the data is significant or not significant, respectively (α = 0.05)

Table 3. Comparison of the fit of the Weibull and simplified Weibull (n = 0.453) model to experimental data for the degradation of patulin (C_0 = 6.40 μM) with added ascorbic acid (C_0 = 1.25 mM) at 25, 35, 45, 55, 65, or 85 ^oC.

Kinetic Model		Kinetic constants		Statistical data			
Weibull	**T (^oC)**	**b (hr^{-n}) (SD)** [1]	**n (SD)**	**R^2**	**SD residuals (Sy.x)**	**Runs test (p-value)**	**Dev. from model** [2]
	25	0.056 (0.0082)	0.458 (0.0151)	0.993	0.0108	0.6224	NS
	35	(0.065 (0.0041)	0.478 (0.0190)	0.988	0.0165	0.4211	NS
	45	0.103 (0.0076)	0.475 (0.0132)	0.995	0.0127	0.8059	NS
	55	0.125 (0.0066)	0.478 (0.0154)	0.994	0.0165	0.6224	NS
	65	0.236 (0.0320)	0.397 (0.0116)	0.994	0.0163	0.5095	NS
	85	0.442 (0.0130)	0.431 (0.0120)	0.999	0.0111	0.2619	NS
Simplified Weibull	**T (^oC)**	**b ($hr^{-0.453}$) (SD)**	**n**	**R^2**	**SD residuals (Sy.x)**	**Runs test (p-value)**	**Dev. from model**
	25	0.06234 (0.0007)	0.453	0.992	0.0109	0.6224	NS
	35	0.07645 (0.0011)	0.453	0.985	0.0179	0.3621	NS
	45	0.1182 (0.0014)	0.453	0.993	0.0152	0.4266	NS
	55	0.1445 (0.0021)	0.453	0.991	0.0193	0.1084	NS
	65	0.2019 (0.0029)	0.453	0.990	0.0210	0.0882	NS
	85	0.4327 (0.0049)	0.453	0.999	0.0108	0.2619	NS

[1] Each value represents the average of three determinations. Standard deviations (SD) are shown in parentheses.

[2] S and NS denote that deviation of the fit of the models to the data is significant or not significant, respectively (α = 0.05)

Figure 1: Degradation of patulin (C_o=6.40 μM) without added AA at (●), 35 (■), 45 (▲), 55(▼), 65 (♦), or 85 ^oC (○) and the fit of the zero-order kinetic model to the experimental data. Each point represents the average of three replicate experiments.

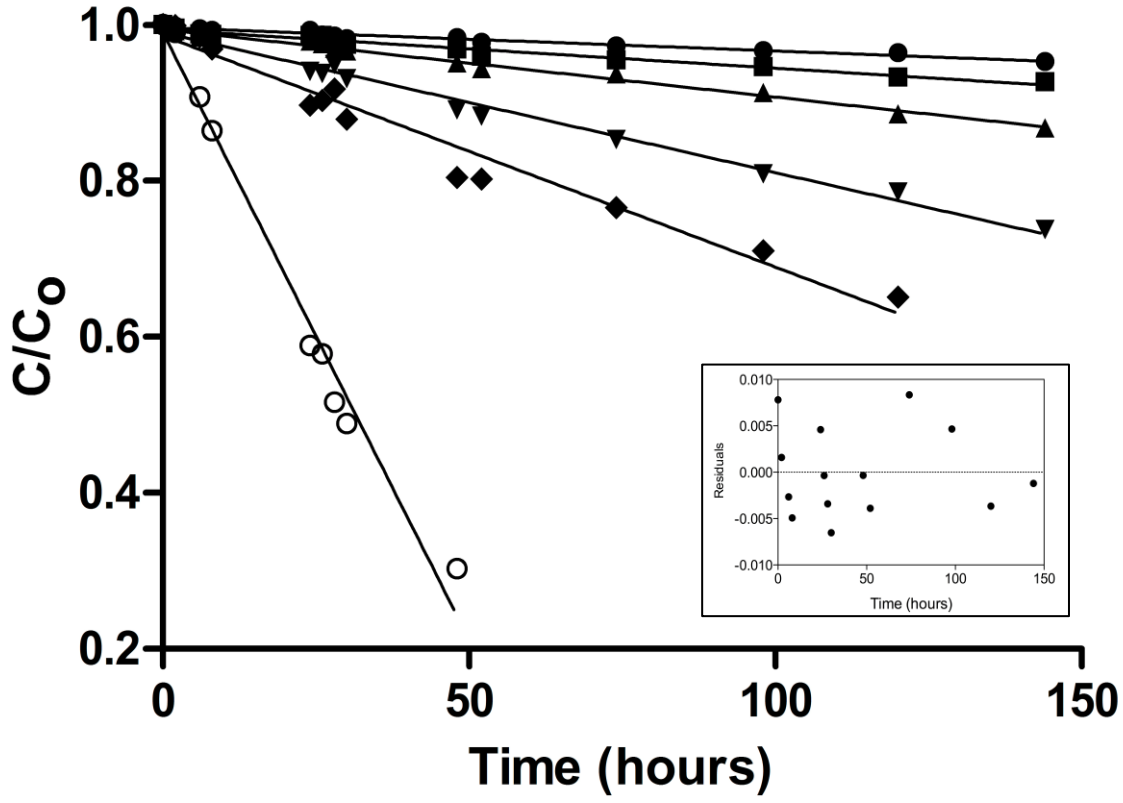
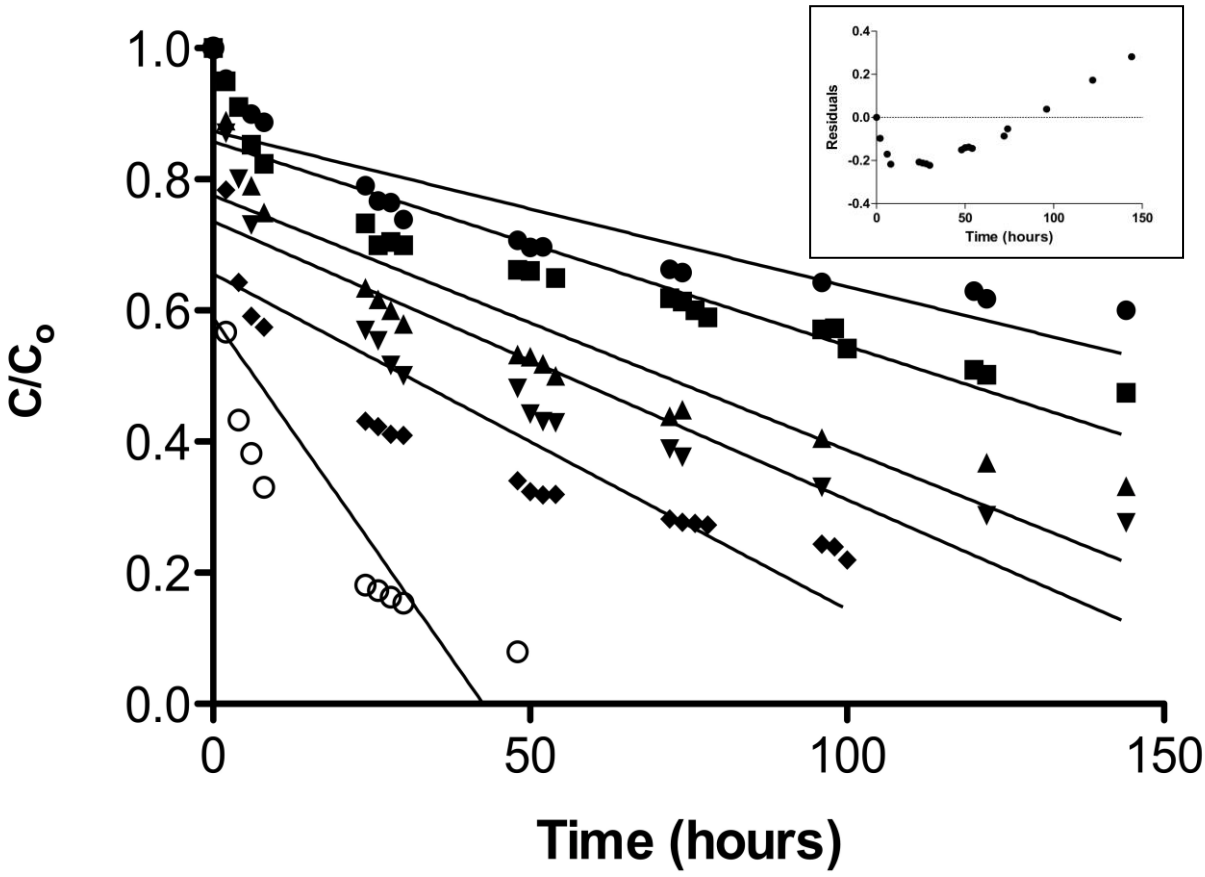
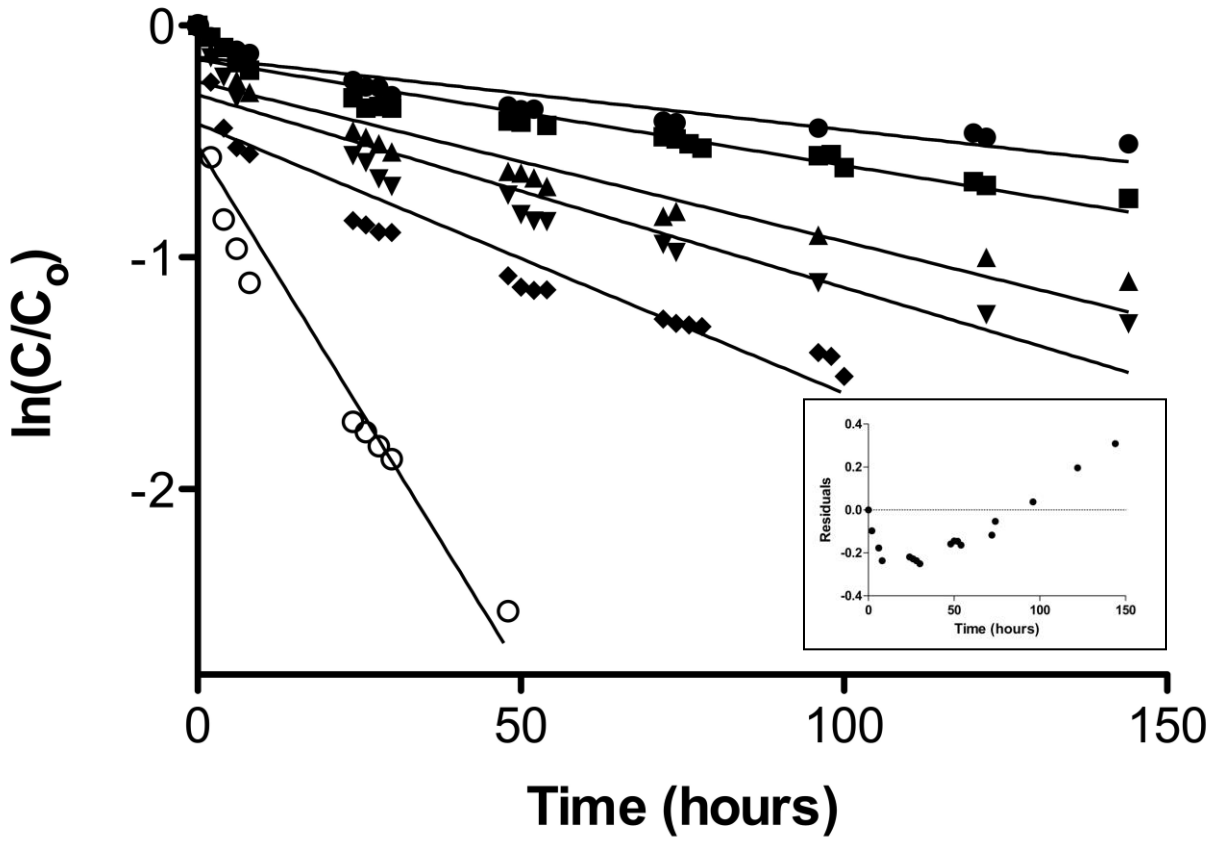


Figure 2: Degradation of patulin (C_o=6.40 μM) with added ascorbic acid (1.25 mM) at (●), 35 (■), 45 (▲), 55(▼), 65 (♦), or 85 ^oC (○) and the fit of the (a) zero-order, (b) first-order, and (c) second-order kinetic model to the experimental data. Each point represents the average of three replicate experiments.

2(a)



2(b)


0
-1
-2
ln(C/C$_o$)
0
50
100
150
Time (hours)
Residuals
0.4
0.2
0.0
-0.2
-0.4
0
50
100
150
Time (hours)

2(c)

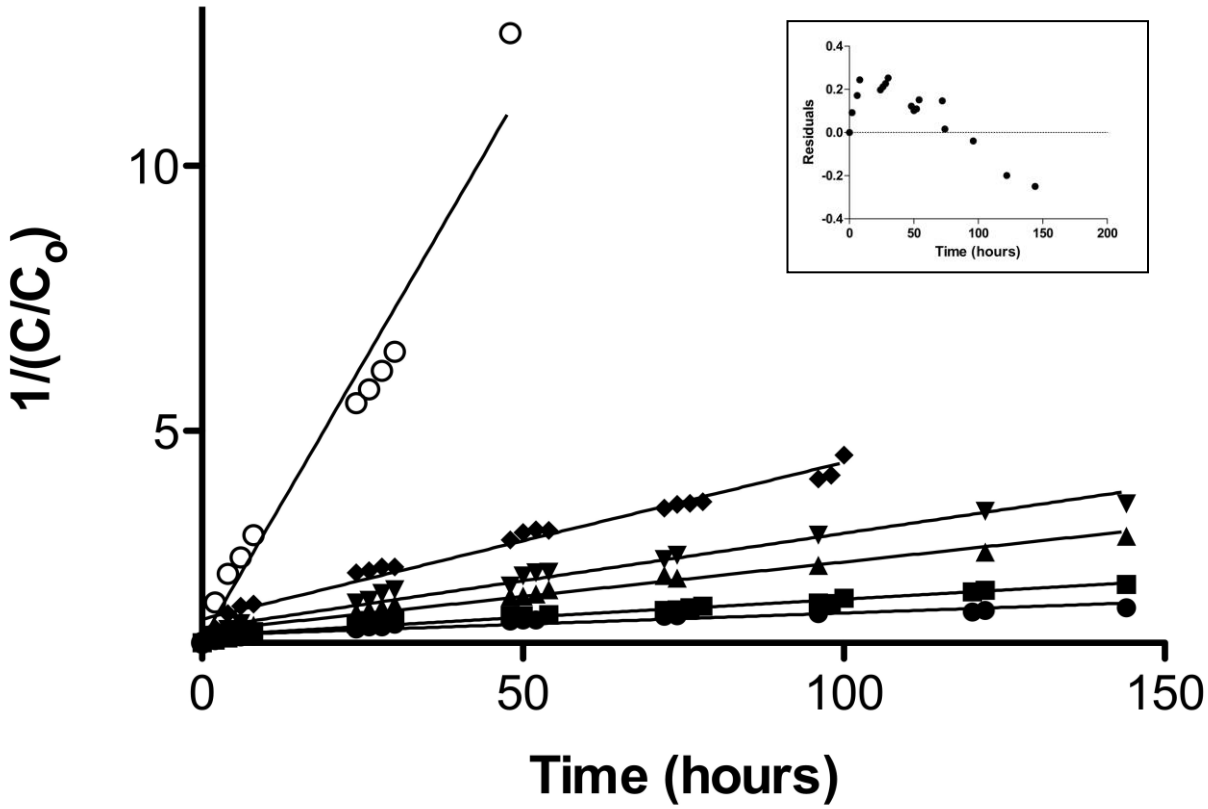
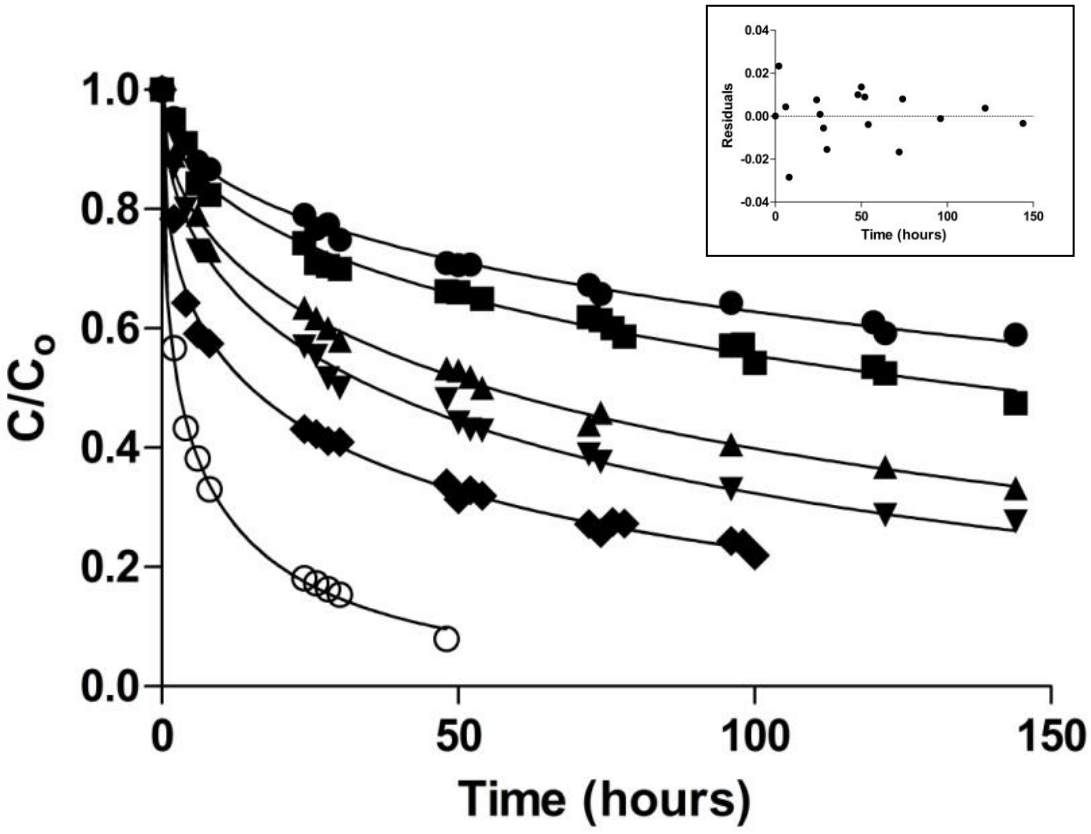

1/(C/C$_o$)
10
5
0
50
100
150
Time (hours)
Residuals
0.4
0.2
0.0
-0.2
-0.4
0
50
100
150
200
Time (hours)

Figure 3: Degradation of patulin (C_o=6.40 μM) with added ascorbic acid (C_o = 1.25 mM) at (●), 35 (■), 45 (▲), 55(▼), 65 (♦), or 85 ^oC (○) and the fit of the (a) Weibull and (b) simplified Weibull (n=0.453) kinetic model to the experimental data. Each point represents the average of three replicate experiments.

3(a)



3(b)

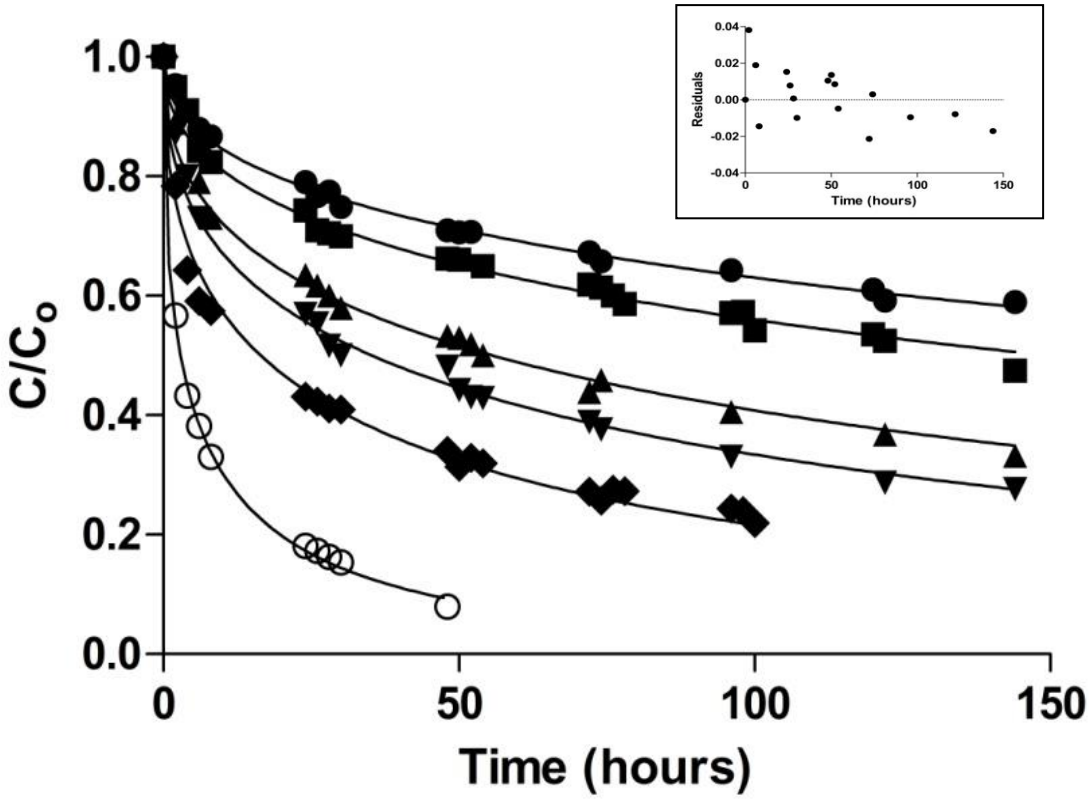

1.0
0.8
0.6
0.4
0.2
0.0
C/Co
0
50
100
150
Time (hours)
Residuals
0.04
0.02
0.00
-0.02
-0.04
0
50
100
150
Time (hours)

Figure 4: Arrhenius plot for the degradation of patulin (C_o=6.40 μM) between 25 and 85 ^oC without (●) or with (▲) added ascorbic acid (1.25 mM). Each point represents the average of 3 determinations ± the standard deviation