This is to certify that the
thesis entitled

KINETIC PARAMETER ESTIMATION FOR DEGRADATION OF ANTHOCYANINS IN GRAPE POMACE

presented by

Dharmendra Kumar Mishra

has been accepted towards fulfillment
of the requirements for the

MASTERS OF SCIENCE	degree in	Biosystems and Agricultural Engineering

Major Professor's Signature

April 11, 2008

Date

KINETIC PARAMETER ESTIMATION FOR DEGRADATION OF ANTHOCYANINS IN GRAPE POMACE

By

Dharmendra Kumar Mishra

A THESIS

Submitted to
Michigan State University
in partial fulfillment of requirements
for the degree of

MASTER OF SCIENCE

Department of Biosystems and Agricultural Engineering

2008

ABSTRACT

KINETIC PARAMETER ESTIMATION FOR DEGRADATION OF ANTHOCYANINS IN GRAPE POMACE

By

Dharmendra Kumar Mishra

Anthocyanins are degraded by heat, especially at temperatures greater than 70°C. Thermal and kinetic parameters were estimated for the degradation of anthocyanins in grape pomace, considered a winery waste that has a potential use as a food ingredient. Nonisothermal experiments were performed, and thermal and kinetic parameters were estimated, using nonlinear regression techniques, for the degradation of anthocyanins in grape pomace at moisture contents of 17, 34, and 42% (wb). Grape pomace was heated in a retort for 8-25 min, at the retort temperature of 126.7 °C in 202 x 214 steel cans. Anthocyanin retention in retorted samples was measured using HPLC. The activation energy E_a, rate of reaction $k_{110^oC,}$ and the moisture parameter b were estimated as 75.34 kJ/g mol, 0.058 min^{-1} and 1.87 $(MC_{wb})^{-1}$. Asymptotic confidence intervals for k_{110^oC} and E_a were (0.055, 0.067) and (32.6,97.9), respectively. Bootstrap 95% confidence interval for k_{110^oC} and E_a were (0.055, 0.066) and (50.9, 100.82) respectively. Prediction bands on the predicted Y (retentions) were computed using the asymptotic and the bootstrap approach. Bootstrap prediction bands were smaller than asymptotic prediction bands. Joint confidence region for thermal parameters were computed using an elliptical approximation and an iterative method.

DEDICATION

To my parents, Ras Bihari Mishra and Sabita Mishra, for their selfless sacrifice and for believing in me and providing all the assistance I needed at all times, to my elder sister Usha Pandey and to my elder brothers Binod Mishra, Ashok Mishra and Ravindra Mishra for their love and affection and to the younger generation. To Balram Singh who taught me the concept of belief.

ACKNOWLEDGEMENTS

I would like to express my utmost gratitude to my advisor, Dr. Kirk Dolan, for all his consistent support and guidance throughout this research period. Dr. Dolan's high motivation and expectations inspired me to achieve beyond my capabilities. Under his mentorship I gained exposure to numerous problem-solving techniques not only in academics but also in other areas. I would like to thank my committee members Dr. Maurice Bennink, Dr. Bradley Marks and Dr. Lijian Yang for their valuable time and effort in the completion of this work and were the source of invaluable advice through our engaging discussions.

I am also grateful to the faculty and staff of Biosystems Engineering as well as Food Science, MSU. I am also thankful to all my friends and fellow graduate students who have provided me the wonderful friendly atmosphere throughout my Master's program and thanks to Dr. Sanghyup Jeyong, for providing me timely assistance. Special thanks to my lab mates at room 129 in Food Science Trout Building for their love and encouragement all the time.

Finally, I would also like to thank my parents, as they are the inspiration for making me what I am today.

TABLE OF CONTENTS

CHAPTER 3

Multi-parameter Estimation and Parameter Confidence Regions for Degradation of Anthocyanins in Grape Pomace

CHAPTER 4

Bootstrap confidence interval for the kinetic parameters for degradation of anthocyanins in grape pomace

LIST OF TABLES

LIST OF FIGURES

Chapter 1

Introduction

1. Introduction

Degradation of the nutraceutical compounds can happen during thermal treatment of the product. For the food to be nutritious and retain the value after processing, one must ensure that the degradation of these compounds is minimal during processing. Special case of anthocyanins in low-moisture and high temperature processed foods, such as grape pomace, has been investigated in this study. Kinetic parameters involved in the degradation model were estimated using the non-linear method of least squares for the non-isothermal heating of grape pomace. Confidence intervals, prediction intervals, joint confidence regions and bootstrap confidence and prediction intervals are discussed in details in subsequent chapters.

1.1 Structure of this Thesis

This work has been divided into three chapters. Each chapter covers a different aspect of the kinetic parameter estimation of the anthocyanins degradation in grape pomace, which is a low- and intermediate-moisture food processed at higher temperature. The three main parts are 1) nonlinear estimation of the two thermal kinetic degradation parameters; 2) joint confidence regions for the two thermal parameters, and multi-parameter parameter estimation when a moisture parameter is added; and 3) bootstrap method for parameter estimation, which is a randomized resampling of the original data to obtain better estimates of the error distribution.

Chapter 1 covers the experimental method used to measure the anthocyanin retention for different time-temperature treatments in a steam retort. It also covers the HPLC analysis, estimation of thermophysical properties and kinetic parameter estimation using the nonlinear regression technique and the confidence interval of the parameters and on predicted Y (retentions).

Chapter 2 covers the multi-parameter model and estimation of the kinetic parameters, such as estimation of parameters at different moisture level of the grape pomace. It also covers the parameter joint confidence region and the sum of square plots to evaluate the ease or difficulty in convergence of the parameters in nonlinear regression.

Chapter 3 is about the bootstrap method to estimate the accurate confidence interval on the kinetic parameters for the degradation of anthocyanins in grape pomace. It also shows the method to calculate the bootstrap confidence interval band and the prediction band on the predicted Y, which provides useful information about the retention of the anthocyanins.

1.2 Background Information on Anthocyanins

Diet and nutrition are increasingly linked with disease prevention and treatment. There has been a great interest in the nutraceuticals in food or as an additive to the food. Grape pomace, which is leftover after the wine making process, is a good source of anthocyanins and hence can be used as an ingredient in food industry. Nutraceutical compounds, such as anthocyanins, are food components known for their health promoting, disease preventing or

medicinal properties. Nutraceuticals not only can act as bright natural colorants (Alexandra Pazmino-Duran and others 2001) but also have potential health benefits, such as antioxidant and anti-inflammatory properties (Wang and others 1999). Anthocyanins have beneficial action against the vascular diseases and contribute towards the reduction of age-related deficits in neurological impairments (Youdim and others 2002).

Most kinetic research in food is conducted on high moisture foods (for example, meats, fruit juice, vegetables and dairy products) at constant temperatures, usually at less than 100°C. Experimental procedures and basic statistical analyses for these products are well established for first order reactions. The 2-step procedure includes plotting the logarithm of concentration versus time at different constant temperatures. The rate constants will be the negative of the slopes. Then plotting the logarithm of the rate constant vs. the reciprocal of temperature will give the activation energy (E_a) from the slope. However, this method is not suitable for low-moisture and high-temperature processed foods because constant temperature can't be attained. There is lack of experimental procedure and statistical analysis of kinetic parameters for components in low moisture and high temperature processed foods such as pastries, breads, baked goods and extruded snacks.

1.3 Structure of Anthocyanins

There are several anthocyanins known to exist (Clifford 2000), and the molecule consists of three parts: the aglycone base on the flavylium nucleus, a group of sugars and a group of acyl acids. The difference among various anthocyanins are: the number of hydroxyl groups, the nature and number of sugars attached to the molecule and position where it is attached, and the number and nature of the acids attached to the sugars attached in the molecule (Mazza and Brouillard 1987). As the number of hydroxyl groups increases, the intensity of blue color increases, and as the number of methoxy group increases, the intensity of redness increases.

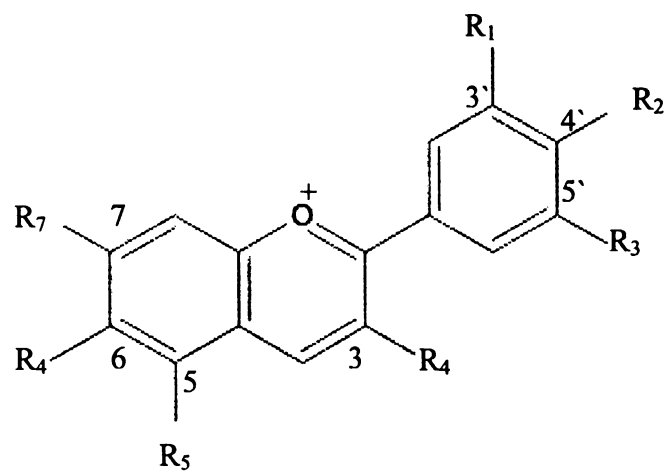


Figure 1.1: The anthocyanin ground structure, flavylium (2-phenylchromenylium) (Wikipedia, 2007)

Table 1.1: Effect of R group on flavylium cation to produce different anthocyanidins (Wikipedia, 2007)

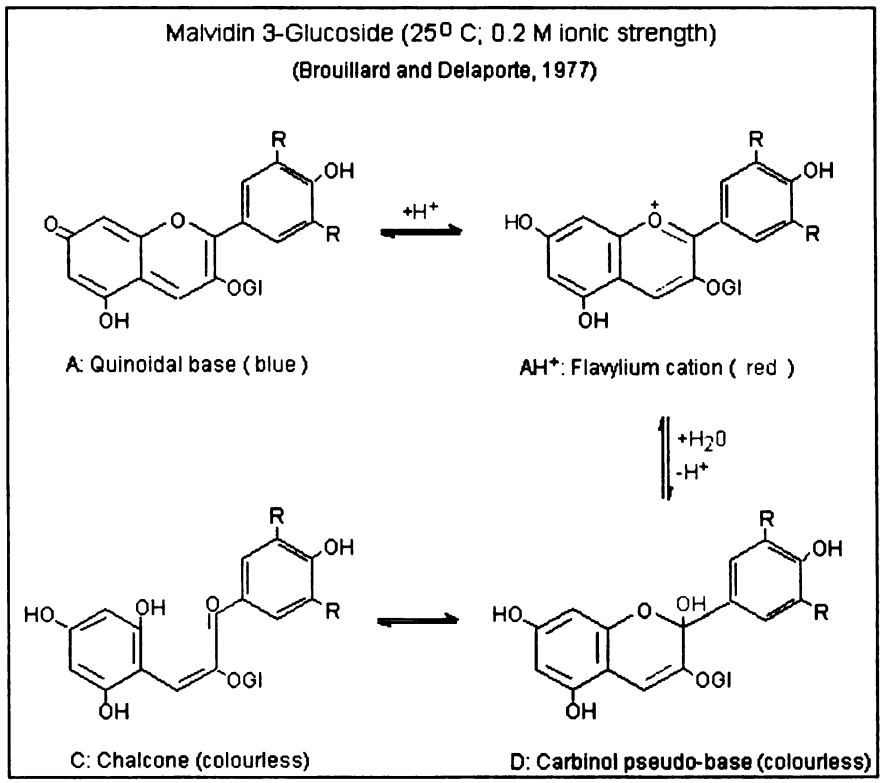
Anthocyanidin	**R_1**	**R_2**	R_3	R_4	**R_5**	R_6	R_7
Aurantinidin	-H	-OH	-H	-OH	-OH	-OH	-OH
Cyanidin	-OH	-OH	-H	-OH	-OH	-H	-OH
Delphinidin	-OH	-OH	-OH	-OH	-OH	-H	-OH
Europinidin	$-OCH_3$	-OH	-OH	-OH	$-OCH_3$	-H	-OH
Luteolinidin	-OH	-OH	-H	-H	-OH	-H	-OH
Pelargonidin	-H	-OH	-H	-OH	-OH	-H	-OH
Malvidin	$-OCH_3$	-OH	$-OCH_3$	-OH	-OH	-H	-OH
Peonidin	$-OCH_3$	-OH	-H	-OH	-OH	-H	-OH
Petunidin	-OH	-OH	$-OCH_3$	-OH	-OH	-H	-OH
Rosinidin	$-OCH_3$	-OH	-H	-OH	-OH	-H	$-OCH_3$

1.4 Stability of Anthocyanins

The pH is the most important factor affecting color stabilization (Mazza, 1987). Structure of anthocyanins exists in four forms (Brouillard, 1982) in equilibrium: the blue quinoidal base A, the red flavylium cation AH+, the colorless pseudobase B and colorless chalcone C.

Cation AH+ yields the quinoidal base A through the loss of a proton. Addition of water to the cation AH+ yields the pseudobase B, and this exists in tautomeric equilibrium with chalcone C. Quinoidal and carbinol pseudobase are unstable. The cation AH+ is the most important form in terms of color and exists in pH below 4. Hence, acidic media are most favorable for the colored anthocyanins.

Figure 1.2: Predominant structural forms of anthocyanins present at different pH levels (Brouillard and Delaporte, 1977)



1.5 Extraction of Anthocyanins and High-Performance Liquid Chromatography

The total extraction of anthocyanins from grape skins has been obtained optimally by superheated liquid extraction using 1:1 (v/v) ethanol-water acidified with 0.8% (v/v) HCL, 120 °C, 30 min, 1.2 ml/min and 80 bar (Luque-Rodriguez and others 2007). Acidified 60% methanol extracts good levels of total anthocyanins from red grape skin using the pressurized liquid extraction (Ju and Howard, 2003). Extraction of anthocyanins from grape pomace was performed, and it was found that the elution with acidified methanol was higher as compared to ethanol and 2-propanol (Kammerer and others 2005). High Performance

Liquid Chromatography (HPLC) with the diode array detector is the most widely used method for the identification and quantification of anthocyanins. However, the quantification of individual anthocyanidins is a challenge, as there are only few anthocyanin standards available commercially. There are several procedures adopted for the extraction of anthocyanins using extraction solvents for the HPLC analysis. Acid hydrolysis of the anthocyanins simplifies its profile, and it can be separated into five different anthocyanidin aglycones (Zhang and others 2004). These anthocyanidins can be easily detected and quantified, as the standards are commercially available.

1.6 Experimental Design

Table 1.2: Experimental Design

Moisture Content (%) wb	Heating time (minute)
42	8 10 12 15 16 17 19 21 23 25
34	15
17	15 16

Grape pomace with moisture content of 42%, 34% and 17% were heated from 8 min to 25 min in steel cans in a steam retort. The 42% moisture content experiments were used for the estimation of rate constant and activation energy, whereas all the moisture content data was used for the estimation of one more parameter i.e. moisture parameter (b). Oxygen radical antioxidant capacity (ORAC) was also measured for the heating time of 58 min and is reported in appendix A.

1.7 References

Alexandra Pazmino-Duran E, Monica Giusti M, Wrolstad RE & Gloria MBA. 2001. Anthocyanins from Oxalis triangularis as potential food colorants. Food Chemistry 75(2):211-216.

Brouillard R. 1982. Chemical structure of anthocyanins. In: Markakis, P., editor). Anthocyanins as Food Colors. New York: Academic Press Inc. . p. 1-38.

Brouillard R & Delaporte B. 1977. Chemistry of anthocyanin pigments. 2. Kinetic and thermodynamic study of proton transfer, hydration, and tautomeric reactions of malvidin 3-glucoside. J. Am. Chem. Soc. 99(26):8461-8468.

Ju ZY & Howard LR. 2003. Effects of Solvent and Temperature on Pressurized Liquid Extraction of Anthocyanins and Total Phenolics from Dried Red Grape Skin. J. Agric. Food Chem. 51(18):5207-5213.

Kammerer D, Gajdos Kljusuric J, Carle R & Schieber A. 2005. Recovery of anthocyanins from grape pomace extracts (Vitis vinifera L. cv. Cabernet Mitos) using a polymeric adsorber resin. European Food Research and Technology 220(3):431-437.

Luque-Rodriguez JM, Luque de Castro MD & Perez-Juan P. 2007. Dynamic superheated liquid extraction of anthocyanins and other phenolics from red grape skins of winemaking residues. Bioresource Technology 98(14):2705-2713.

Mazza GaRB. 1987. Colour stability and structural transformations of cyanidin 3,5-diglucoside and four 3-deoxyanthocyanins in aqueous solutions. Journal of Agricultural and Food Chemistry:422-426.

Wang H, Nair MG, Strasburg GM, Chang YC, Booren AM, Gray JI & DeWitt DL. 1999. Antioxidant and Antiinflammatory Activities of Anthocyanins and Their Aglycon, Cyanidin, from Tart Cherries. J. Nat. Prod. 62(2):294-296.

Wikipedia. 2007. Anthocyanin. http://en.wikipedia.org/wiki/Anthocyanin.

Youdim KA, McDonald J, Kalt W & Joseph JA. 2002. Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults. The Journal of Nutritional Biochemistry 13(5):282-288.

Zhang Z, Kou X, Fugal K & McLaughlin J. 2004. Comparison of HPLC Methods for Determination of Anthocyanins and Anthocyanidins in Bilberry Extracts. J. Agric. Food Chem. 52(4):688-691.

CHAPTER 2

Mishra, D.K, Dolan, K.D., Yang L. 2008. Confidence intervals for modeling anthocyanin retention in grape pomace during nonisothermal heating. J. Food Sci. 73(1):E9-E15.

Abstract

Degradation of nutraceuticals in low- and intermediate-moisture foods heated at high temperature (>100°C) is difficult to model because of the nonisothermal condition. Isothermal experiments above 100°C are difficult to design, because they require high pressure and small sample size in sealed containers. Therefore, a non-isothermal method was developed to estimate the thermal degradation kinetic parameters of nutraceuticals, and determine the confidence intervals for the parameters and the predicted Y (concentration). Grape pomace at 42% moisture content (wb) was heated in sealed 202 x 214 steel cans in a steam retort at 126.7 °C for > 30 min. Can center temperature was measured by thermocouple and predicted using Comsol software. Thermal conductivity (k) and specific heat (C_p) were estimated as quadratic functions of temperature using Comsol® and nonlinear regression. The k and C_p functions were then used to predict temperature inside the grape pomace during retorting. Similar heating experiments were run at different time-temperature treatments from 8 to 25 min for kinetic parameter estimation. Anthocyanin concentration in the grape pomace was measured using HPLC. Degradation rate constant (k_{110^oC}) and activation energy (E_a) were estimated using nonlinear regression. The thermophysical properties estimates at 100°C were k = 0.501 W/m°C, C_p = 3600. J/kg°C and the kinetic parameters were k_{110^oC} = 0.0607 min^{-1} and E_a=65.32 kJ/mol. The 95% confidence intervals for the parameters, and the confidence bands and prediction bands for anthocyanin retention were plotted. These methods are useful for thermal processing design for nutraceutical products.

2.1 Introduction

Diet and nutrition are increasingly linked with disease prevention and treatment. Nutraceutical compounds, such as anthocyanins, are food components known for their health promoting, disease preventing or medicinal properties. Nutraceuticals not only can act as bright natural colorants (Alexandra Pazmino-Duran and others 2001) but also have potential health benefits, such as antioxidant and anti-inflammatory properties (Wang and others 1999). Anthocyanins have beneficial action against vascular diseases and contribute towards the reduction of age-related deficits in neurological impairments (Youdim and others 2002). Grape pomace is a rich source of anthocyanins. It is the solid part of the fresh grapes, and consists of skins and the seeds. Grape pomace, a waste product from the winemaking process, is used for composting or cattle feed or may end up in landfills.

Many solid foods could be enriched with anthocyanins, such as extruded foods, baked goods, cereals, etc. However, anthocyanins are unstable at high temperatures. Food processing usually involves the use of heat and other physical methods, which may destroy these beneficial compounds. It is therefore important to study the effect of such processing on the nutritional quality of pomace. Developing mathematical models that take into account the important process variables (T, pH, time) that influence thermal degradation of thermosensible compounds will be a useful tool for process design.

Anthocyanins are sensitive to temperature, especially above 70° C (Markakis and others 1957). Anthocyanin obtained from concord grape was processed, and it was found that the pigment loss, analyzed using spectrophotometry, was 32% at 77 °C, 53% at 99 °C and 87% at 121 °C (Sastry, 1953). Thermal degradation of anthocyanins was also studied for concord grapes (Calvi and Francis, 1978). Degradation of anthocyanins was reported while processing blueberries into juice and concentrate and it was found that different classes have varying susceptibility to degradation with different unit operations (Skrede G, 2000). The rate of degradation of anthocyanins is time and temperature dependent. Temperature dependence of the degradation of anthocyanins has been shown to follow first-order kinetics (Cemeroglu and others 1994); (Kirca and Cemeroglu, 2003) and can be modeled using the Arrhenius relationship (Ahmed and others 2004). The degradation kinetics of anthocyanins in blood orange juice was studied (Kirca and Cemeroglu, 2003) and the activation energies for solid content of 11.2 to 69 °Brix were found to be 73.2 to 89.5 kJ/mol.

Although many studies have determined the thermal degradation kinetics of anthocyanins in solution, very few studies have investigated anthocyanin degradation in solids. Extrusion of corn meal (extruder die temperature 130°C) with grape juice concentrate and blueberry concentrate showed up to 74% anthocyanin degradation in the extruded cereal (Camire and others 2002). Extrusion of corn meal with dehydrated fruit powder (blueberry, cranberry, Concord grape and raspberry) in proportions of 84.3, 14.3, 0.4 and 1.0%

(cooking temperature up to 130 °C) showed up to 90% decrease of anthocyanins for all dehydrated fruits used except raspberry (Camire and others 2007). Although degradation of anthocyanins was reported, kinetic parameters were not estimated.

Estimation of the parameters for an isothermal process, such as kinetic parameters for anthocyanin degradation in juices and concentrates, is mathematically straightforward. Anthocyanins have been found to follow the first-order reaction kinetics (Cemeroglu et al., 1994); (Kirca and Cemeroglu, 2003) and can be modeled using the Arrhenius relationship (Ahmed et al., 2004):

$$k = k_r e^{\left(\frac{-E_a}{R}\left(\frac{1}{T} - \frac{1}{T_r}\right)\right)} \quad (1)$$

For isothermal processes, taking the logarithm of both sides of Eq. (1) simplifies the problem. However, thermal processing of solid foods is not isothermal, but instead shows time-varying temperature. Therefore, in kinetic modeling for non-isothermal processes, the exponential term has the time-temperature history as an integral and hence requires nonlinear regression techniques (Dolan and others 2007).

Experimental procedure for the kinetics in high moisture content foods (for example, meats, fruit juice, vegetables and dairy products) is well established. The 2-step procedure for kinetics in these foods includes plotting the logarithm of concentration versus time at different constant temperatures. The negative of the

slope of this line is the degradation rate constant. Then plotting the logarithm of the degradation rate constant vs. the reciprocal of temperature will give the activation energy (E_a) from the slope. However, this method is not suitable for low- and intermediate-moisture and high-temperature processed foods because constant temperature cannot be attained. There is lack of experimental procedure and statistical analysis of kinetic parameters for components in low moisture and high temperature processed foods such as pastries, breads, baked goods and extruded snacks.

Some researchers have proposed small samples, such as 13.5-mm diameter test tubes (Dolan and Steffe, 1989) with gelatinized starch solutions, and the use of transient heat-transfer theory to predict the temperature within the sample. (Rob van den Hout, 1999) used 2-mm thick containers for isothermal heating of soy flour so that temperature variation over the thickness could be neglected.

Several non-isothermal methods to estimate kinetic degradation parameters have been reported. The method of Paired Equivalent Isothermal Exposures (PEIE) was applied to microbial survival data from retort experiments of canned pea puree (Welt, 1997). PEIE was used to estimate the Arrhenius parameters.

The kinetic model for thiamine destruction in pea puree (Nasri and others 1993) was developed using nonlinear regression for the parameter estimation

and the jackknife method for the estimation of the experimental error. However, in their study the confidence interval of the parameters and the confidence interval for predicted Y (microbial retention) was not reported.

In summary, no standard method was found in the literature, for the dynamic kinetic parameter estimation of low- and intermediate-moisture foods. There is a lack of nonlinear regression techniques for analysis of unsteady-state conduction-heated foods. In the context of non-isothermal processing, the objective of this study was to establish an experimental procedure and mathematical and statistical analysis to (1) estimate kinetic parameters for thermal degradation of nutraceuticals, and (2) to provide confidence and prediction bands for the nutrient retention in low- and intermediate-moisture processed foods, using anthocyanins in grape pomace as a model nutraceutical.

2.2 Mathematical Model and Simulation

2.2.1 Thermal Parameter Estimation

A finite element method was used to solve the heat transfer equations and to predict the center temperature of the can. Finite element approximates a PDE with finite number of unknown parameters based on the geometry. Actual dimensions of the can (radius 0.027m, and height 0.073m) were used to create the finite cylinder geometry in CAD provided in Comsol. The retort time-varying convective boundary condition (approximately 5-min come-up time, then constant retort temperature (126.7 °C)) with constant h was used on all surfaces. A second-order polynomial was used to model thermal conductivity and specific heat of grape pomace as a function of temperature (Kenneth J. Valentas, 1997), where T was in Centigrade:

$$k(T) = a + bT + cT^2 \tag{2}$$

$$C_p(T) = a' + b'T + c'T^2 \tag{3}$$

Comsol is a finite commercial finite-element software package that uses Delaunay algorithm to create Mesh elements. Mesh parameters determine the element size and element distribution. Comsol mesh parameters used in this study were:

Maximum element size scaling factor: this is the scaling factor for maximum allowed element size.

Element growth rate: determines the maximum growth rate of element from a region with smaller elements to a region with larger elements.

Mesh curvature factor: determines the size of an element near the boundary depending on the curvature of the geometry.

Mesh curvature cut off: a positive number that prevents formation of many elements around small curvature of the geometry.

Moreover, the mesh parameters determine the accuracy of the result and the computation time. Smaller mesh size might provide better estimates, but it may increase the computation time significantly. Hence, depending on the problem and the geometry, mesh size can be optimized to reduce the computation time and to get better estimates of the parameters. The values of maximum element size scaling factor, element growth rate, mesh curvature factor and mesh curvature cutoff in the present study were 1.5, 1.5, 0.5, 0.02, respectively.

Initial guesses of the six parameters (a, b, c, a′, b′ and c′) were fed into Matlab. The time step was 30 sec and cooling temperatures were calculated until the center temperature of can was below 80 °C. Initial values of k and C_p were computed by Matlab and fed into Comsol to predict the center temperature of the can. Sum of squares (SSQ) of predicted and measured can temperature were minimized by nonlinear regression (nlinfit in Matlab) to obtain the final estimates of the parameters. Thermal diffusivity was calculated using the following equation:

$$\alpha(T) = \frac{k(T)}{\rho\, Cp(T)} \tag{4}$$

2.2.2 Kinetic Parameter Estimation

Predicted mass average concentration of anthocyanins was calculated per the following equation:

$$\left(\frac{\bar{C}}{C_o}\right)_{pred} = 2\iint e^{-k_r \int_0^t e^{\frac{-E_a}{R_g}\left(\frac{1}{T(r,z,t)} - \frac{1}{T_r}\right)} dt}\, r\, dr dz \tag{5}$$

Variables r and z were normalized, so the limits for the r and z integral were from 0 to 1.

The estimates of the thermophysical properties were used in Comsol to compute *T(r, z, t,)* for the can dimension based on $\alpha(T)$, T_i, and T_∞. Due to the symmetry of the can, nine points in $1/8^{th}$ of the can were chosen. Gauss integration was preferred over the trapezoidal method for the spatial integral, as Gauss provides more accuracy with minimum number of nodes. Nodes were chosen at 3 r, and 3 z locations, giving the total of 9 points as shown in appendix C. Therefore, the numerical solution to Eq. (5) was

$$\left(\bar{C}(k_r,t_n)/C_o\right)_{pred} \cong 2\sum_{j=1}^{3}\sum_{i=1}^{3}\exp\left[-k_r\beta\left(r_{ij},z_{ij},t_n\right)\right]r_{ij}w_iw_j \qquad (6)$$

where the time-temperature history, β is the time integral in Eq. (5) Because Gauss integration is not convenient when integral limits change, the trapezoidal method (trapz in matlab) was used for the integration of the time-temperature history (Dolan and others 2007):

$$\beta(r,z,t) \cong \sum_{n=0}^{N-1}\frac{\Delta t}{2}\left\{\exp\left[\frac{-E_a}{R_g}\left(\frac{1}{T(r,z,t_{n+1})}-\frac{1}{T_r}\right)\right]+\exp\left[\frac{-E_a}{R_g}\left(\frac{1}{T(r,z,t_n)}-\frac{1}{T_r}\right)\right]\right\} \qquad (7)$$

2.2.3 Sum of Squares of Errors

Sum of Squares was minimized by nonlinear regression to obtain the estimated values of k_r and E_a.

$$SSQ=\sum_{i=1}^{n}\left[\left(\bar{C}/C_o\right)_{obs,i}-\left(\bar{C}/C_o\right)_{pred,i}\right]^2 \qquad (8)$$

2.2.4 Confidence Intervals

To compute asymptotic confidence/predicted intervals, two commands in Matlab® were used, which are:

for parameters: nlparci(beta, residuals, Jacobian)

for predicted Y value: nlpredci(model, x, beta, residuals, Jacobian, simultaneousoption, predictionoption)

nlparci(beta,resid,J) returns the 95% asymptotic confidence interval CI on the nonlinear least squares parameter estimates "beta". 95% asymptotic confidence intervals for the two parameters were calculated using nlparci, and the 95% asymptotic prediction interval was calculated for the predicted Y using nlpredci. Asymptotic prediction intervals (95%) were calculated by (Montgomery, 2006)

$$\text{prediction width}_i = \sqrt{(\text{confidence width}_i)^2 + (t_{\alpha/2,n-p} * RMSE)^2} \quad (9)$$

For researchers without access to Matlab, all of these confidence and prediction intervals can be computed in Excel using equations found in standard statistical texts (Seber, 1989).

2.2.5 Standard Error and Correlation Coefficient

Variance-covariance matrix gives the information about the standard error σ_i of parameters (Van Boekel, 1996). σ_i is the square root of the corresponding diagonal of the symmetric parameter

$$\mathbf{cov(a)} = (\mathbf{X}^{\mathrm{T}}\mathbf{X})^{-1}(MSE) = \begin{pmatrix} \sigma^2_{k_r} & \sigma_{k_r E_a} \\ \sigma_{k_r E_a} & \sigma^2_{E_a} \end{pmatrix} \tag{10}$$

where, **X** is the Jacobian

$$\mathbf{X} = \begin{pmatrix} \left(\frac{\partial Y_1}{\partial k_r}\right) & \left(\frac{\partial Y_1}{\partial E_a}\right) \\ \vdots & \vdots \\ \left(\frac{\partial Y_n}{\partial k_r}\right) & \left(\frac{\partial Y_n}{\partial E_a}\right) \end{pmatrix} \tag{11}$$

The correlation coefficient $\rho_{k_r E_a} = \sigma_{k_r E_a} / \left(\sigma_{k_r} \sigma_{E_a}\right)$ varies from -1.0 to 1.0.

The parameter estimation process with higher values of $\left|\rho_{k_r E_a}\right|$ indicate more difficulty in the estimation process.

2.3 Methods and Materials

2.3.1 Sealing and Retorting of Grape Pomace

Concord grape pomace was obtained from St. Julian Winery in Paw Paw, MI and stored at -15°C for lab analysis. Grape pomace was thawed at room temperature and kept ready to fill in cans. Steel cans (radius 0.027m, and height 0.073m) were filled with the grape pomace. Density of grape pomace in each can was approximately 640 kg/m^3 at 42% (wb) moisture content and sealed under vacuum (20 mm Hg) (Dixie Seamer, Athens, Georgia). Cans were fitted with needle thermocouples (Ecklund-Harrison Technologies Inc., Fort Myers, Florida) at the geometric center of the can to obtain the center temperature to be used later for thermophysical properties parameter estimation. Uniform initial temperature of the product in each can was recorded. These cans were processed in a rotary steam retort (FMC Steritort Laboratory Sterilizer) in triplicates. Retort steam temperature was 126.7°C. Cans were not rotated and were placed stationary on the bottom of the retort throughout heating. For thermal parameter estimation, heating time >30 min was used for 6 cans with two replicates. Processing time for kinetic parameter estimation was selected based on the degradation of the anthocyanins. Three replicates each for 11 times ranging from 8-25 min were used for estimating kinetic parameters. Two thermocouples were fixed in the retort to record the retort steam temperature during processing. Cans were cooled, such that the center temperature was not more than 40 °C, with water at 25 °C in retort. The grape pomace from each can

was then removed and mixed for 40 s in a mixer/blender (Cuisinart® Mini Prep plus, East Windsor, NJ) to achieve uniform sampling (coefficient of variance < 10%). The samples were then kept in plastic bags at –12°C for further analysis.

2.3.2 Extraction of Anthocyanins from Grape Pomace

Grape pomace (15g) was mixed with solution containing 2 N HCL (50 ml of methanol + 33 ml of water + 17 ml of 37% HCL) and sonicated for 20 min and then filtered through the vaccum filter using No. 4 whatman filter paper (Zhang and others 2004). The filtrate was evaporated in a rotary evaporator at 35°C and the retained anthocyanins in the flask were diluted with 25 ml of water and kept for HPLC analysis.

2.3.3 Anthocyanins measurement

Anthocyanins were extracted, and the values were measured in the laboratory using the HPLC method. $\overline{C}$, observed mass-average concentration of anthocyanins in each can was measured. Observed retention was computed as $\left(\overline{C}/C_o\right)_{obs}$. $\overline{C}$ was computed as the total area under the peaks for all anthocyanidins.

2.3.3.1 Separation of Anthocyanins and Polyphenolics

Extracted anthocyanins solution (1 ml) was mixed with 0.25 ml of 12.1 N HCL and 0.25 ml of water (Skrede G, 2000) in a screw-cap test tube and heated for 30 min in a boiling water bath. The hydrolysate was cooled in an ice bath and

then centrifuged at 2500 rpm for 10 min. The centrifuged hydrolysate was applied to C–18 Sep-Pak cartridge which was previously activated with 5 ml ethyl acetate, 5 ml acidified (0.01% HCl) methanol, and 5 ml acidified (0.01% HCL) water respectively. The absorbed sample in the cartridge was then washed with 5 ml of acidified (0.01% HCL) water. Polyphenolics were eluted using ethyl acetate (5 ml). Anthocyanins were recovered from the cartridge using acidified (0.01% HCL) methanol, and the resulting solution was evaporated to near dryness in a Heidolph Laborota 4002 rotary evaporator at 35°C. The final concentrate was taken up in 1 ml of acidified methanol.

2.3.3.2 HPLC system

Waters Breeze software 3.30 (Waters Corp., Milford, Mass., USA) software was used along with Waters 2487 dual wavelength absorption detector, 1525 binary HPLC pump and a 717 plus autosampler. Samples were filtered through 25 mm nylon 0.2 μm filters and injected through the autosampler, and peaks were identified according to their absorbances at 520 nm.

2.3.3.3 Anthocyanidins Separation

Anthocyanidins were separated using Agilent Eclipse XDB C-18 column (5 μm) 4.6 x 250 mm i.d. Solvent A was 100% acetonitrile, and Solvent B was a mixture of 1% phosphoric acid, 10% acetic acid and 5% acetonitrile (v:v:v) in water. Linear gradient from 0 to 30% A was used for 30 min.

2.4 Results

The HPLC chromatograms for raw grape pomace and for grape pomace retorted 14 min at 126.7°C are shown in Figs. 2.1 and 2.2, respectively. The separation was very clear, and individual anthocyanidins were identified well. The degradation of the anthocyanins can be seen in Fig. 2.2; the peaks in the chromatogram are lower for the retorted grape pomace than for the raw grape pomace.

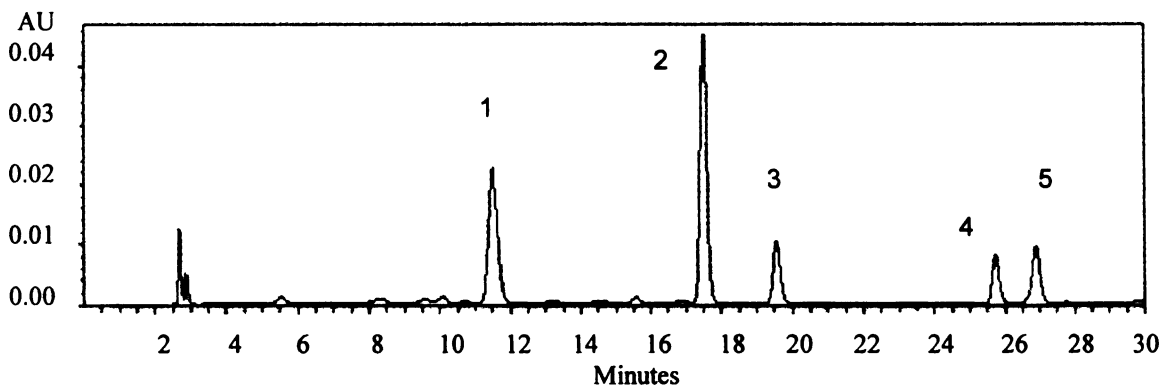


Figure 2.1: HPLC chromatogram for the raw grape pomace. Peak identification 1, delphinidin; 2, cyanidin; 3, petunidin; 4, peonidin; 5, malvidin.

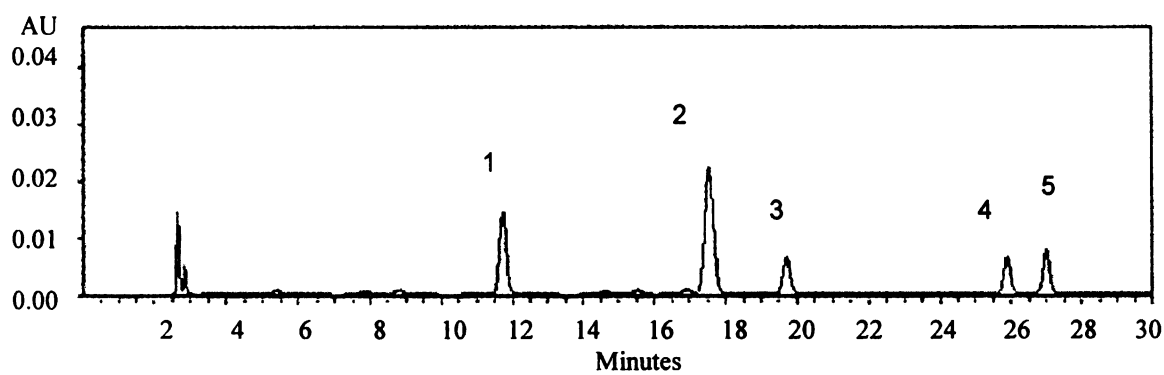


Figure 2.2: HPLC chromatogram for the retorted grape pomace at 126.7 °C for 14 minutes. Peak identification 1, delphinidin; 2, cyanidin; 3, petunidin; 4, peonidin; 5, malvidin.

Table 2.1 shows the anthocyanin retention ratios for the 42 samples. The anthocyanin content of the raw grape pomace was, $\bar{C}_0 = 104.7\ mg/ml$ based on the standard anthoyanin (Pelargonidin). The repeatability of the HPLC assay was within 4% of the anthocyanin concentration.

2.4.1 Thermal Parameter Estimation

Figure 2.3 shows the observed temperature (provided in appendix D) vs. the predicted temperature (Comsol). The root mean square error was 1.35°C.

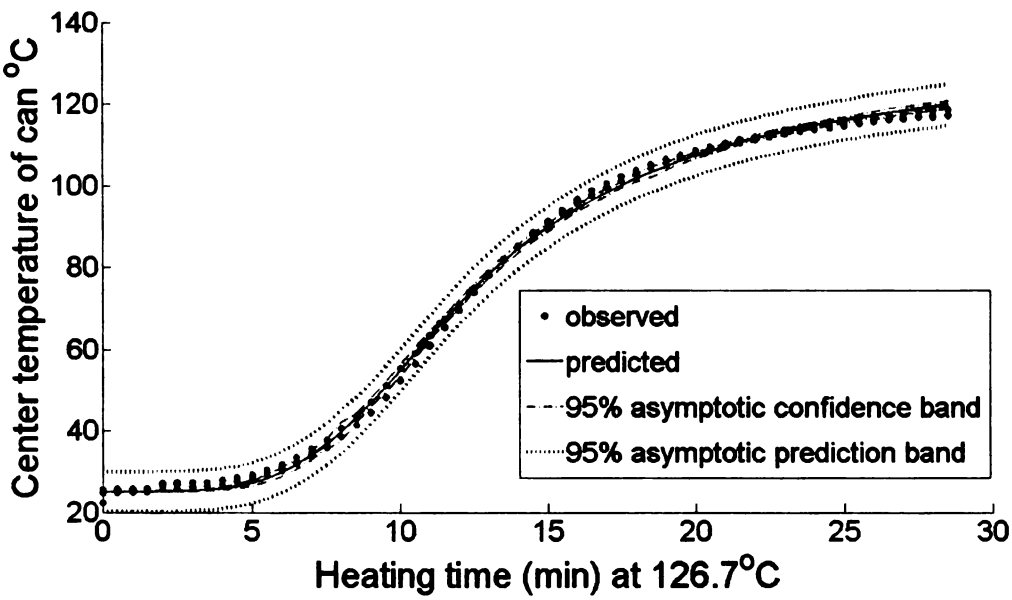


Figure 2.3: Example plot of observed and predicted center temperature profiles from Comsol (triplicate runs) in canned grape pomace

For 42% moisture (wb) grape pomace, the thermal conductivity (k) and specific heat (C_p) were estimated as given in Table 2.2. For example, the values calculated at 100°C are, k = 0.501 W/m°C, C_p = 3600. J/kg°C and Thermal Diffusivity = 2.007 x 10^{-7} m^2 /s.

Table 2.1: Heating time and measured anthocyanin retention for grape pomace at 42% (wb) moisture content at 126.7 °C retort temperature for 33 heated cans

Sample number	Heating time (min.)	Retention of anthocyanins
1	0	1.117
2	0	0.855
3	0	0.855
4	0	0.883
5	0	1.029
6	0	0.984
7	0	1.007
8	0	1.110
9	0	1.159
10	8	0.837
11	8	0.733
12	8	0.804
13	10	0.688
14	10	0.684
15	10	0.686
16	12	0.698
17	12	0.744
18	12	0.714
19	14	0.565
20	14	0.515
21	14	0.409
22	15	0.498
23	15	0.507
24	15	0.599
25	16	0.341
26	16	0.285
27	16	0.433
28	17	0.301
29	17	0.309
30	17	0.305
31	19	0.212
32	19	0.241
33	19	0.227
34	21	0.234
35	21	0.188
36	21	0.249
37	23	0.218
38	23	0.222
39	23	0.213
40	25	0.170
41	25	0.155
42	25	0.145

Table 2.2: Thermal property parameters for quadratic model for grape pomace at 42% (wb) moisture content

Parameter	1st Parameter	2nd parameter	3rd parameter	RMSE of temperature
k (Thermal conductivity, W/m °C) Eq. 2	a = 0.32717	b= 0.00174	c = -3.786e-008	1.35 °C
C_p (Specific heat, J/kg °C) Eq. 3	a′ = 2865.2	b′ = -0.9434	c′ = 0.08316	

2.4.2 Kinetic Parameter Estimation

The kinetic parameters for the anthocyanin degradation obtained from the nonlinear regression technique are shown in Table 2.3:

Table 2.3: Degradation Kinetic parameters for anthocyanin degradation in grape pomace at 42% (wb) moisture content

Parameter	No. of Data	Parameter Estimates	Standard Error	$\rho_{k_r E_a}$ Correlation coefficient (ref. temperature)	95% asymptotic confidence interval	RMSE $\left(\bar{C}/C_o\right)$
k_{110^oC}	42	0.0606 min^{-1}	0.003	-0.12 T_r =110°C	(0.055, 0.067)	0.084
E_a		65.32 kJ/g mol	16.15		(32.7, 97.9)	

Figure 4 shows the 95% asymptotic confidence band and 95% prediction band for mass average retention of anthocyanins. The correlation coefficient is –

0.12. The confidence band (CB) is the region where 95% of the regression lines are expected to be, so it is not uncommon for large number of data observations to fall outside this region. The prediction band (PB) is the region where 95% of the data are expected to be. If more data were collected, we expect ~5% of all the data would fall outside the PB.

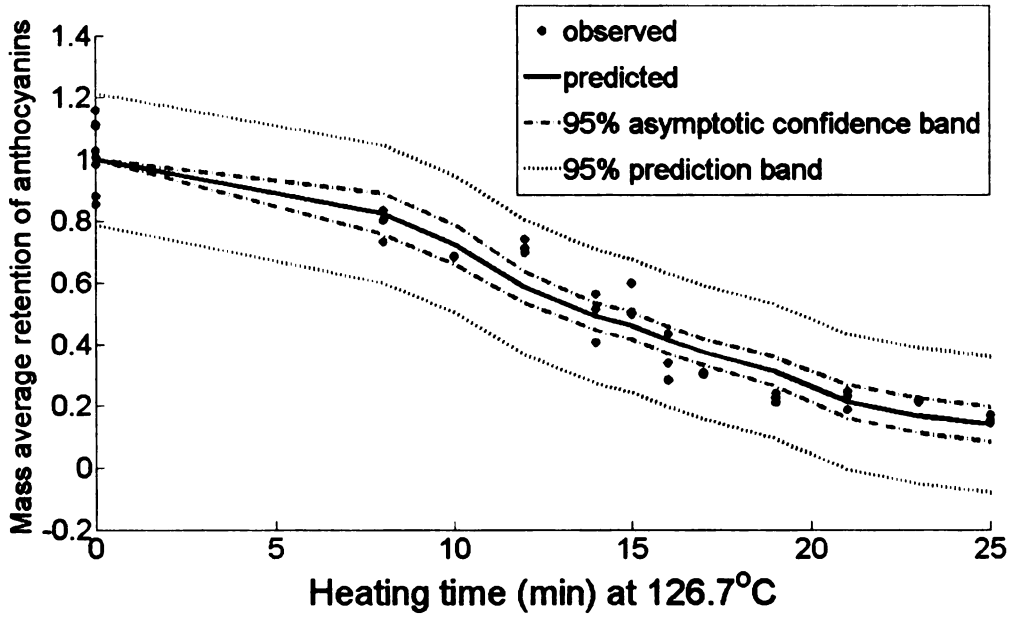


Figure 2.4: Mass average retention of anthocyanins in grape pomace heated in 202 x 214 cans at retort temperature of 126.7 °C

The RMSE (Root Mean Square Error) = 0.084 is ~9% of the total scale of 1.0 (Fig. 4), showing a good fit. The CI for E_a is proportionally larger than the CI for $k_{110^\circ C}$ (Table 3), but the size of the CB and PB for nutrient retention is reasonably small (Fig. 2.4). Processors would be more interested in the PB of the retention, rather than CI of the parameters. For example, using the lower bound

of the PB in Fig. 4, one can predict that only 2.5% of one lot of cans heated for 10 min at 126.7°C will have retention ≤50%, while the remaining 97.5% of cans will have retention >50%.

Residual plots were also plotted to check for the absence of trends or correlations between the parameters. The residuals are scattered around the center (Fig. 2.5) and show a nearly normal distribution. This plot confirms a good fit of the Arrhenius model to the degradation of anthocyanins in grape pomace.

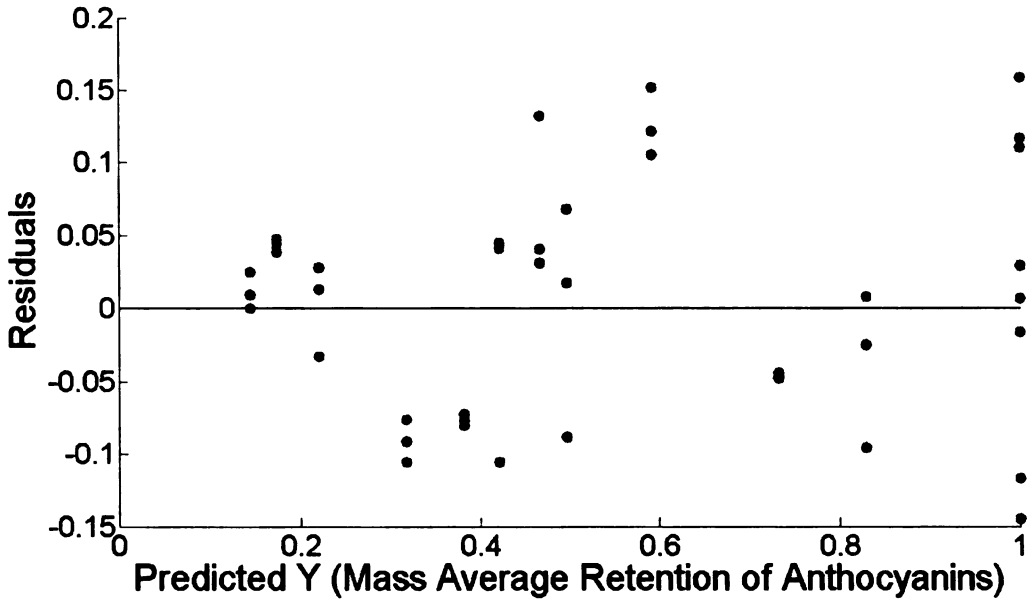


Figure 2.5: Residual plot of the mass average retention of anthocyanins for cans heated at retort temperature of 126.7 ^{o}C

The activation energy for the anthocyanin degradation obtained from this study was comparable to the values for anthocyanin degradation in solution found in the literature. Other researchers reported that the activation energy ranged from 25.0 kJ/g-mol to 85 kJ/g-mol, and the rate of reaction (k_0) ranged

from 5.62 x 10^{10} $min^{-1}$to 3.64 x 10^{25} min^{-1} (Cemeroglu et al., 1994; Ahmed et al., 2004; St. S. Tanchev, 1974). The value of rate of reaction, k_0, found in the present study was 4.9 x 10^7 min^{-1}. As expected, the degradation rate k_0 in the intermediate-moisture food was lower than the k_0 in liquids.

Kinetic parameters for the anthocyanin degradation obtained from extrusion of grape pomace with wheat flour in the ratio 1:3 (Lai, 2003) were comparable with the parameters estimated in this study. The activation energy was 76.0 kJ/g-mol (Lai, 2003) versus 65.32 kJ/g-mol in the present study, and the rate of reaction (k_{80}) was 0.049 min^{-1} (Lai, 2003), versus 0.011 min^{-1} in the present study (converted from Table 3 using Eq. 1). Lai (2003) heated her samples at atmospheric pressure up to 140°C, allowing the moisture content to decrease during heating, and then corrected the data. In the present study, heating occurred at constant pressure and constant moisture. The reasonable agreement of the estimates between two studies that were conducted with different methods is a strong indication that the estimates are close to the true values.

2.4.3 Plot of Sum of Squares

The 3-D surface plots of the sum of squares (fig. 2.6) provide information about the nature of standard error and confidence intervals, and the relative ease of convergence of the nonlinear regression routine. Figure 6 shows that the surface is shallower along the E-axis than along the $k_{110°C}$ axis, causing the

standard errors and confidence intervals for E to be proportionately larger than that for $k_{110°C}$, consistent with the CI results in Table 2.2. Better convergence can be obtained if the curve shows steeper change along the E axis.

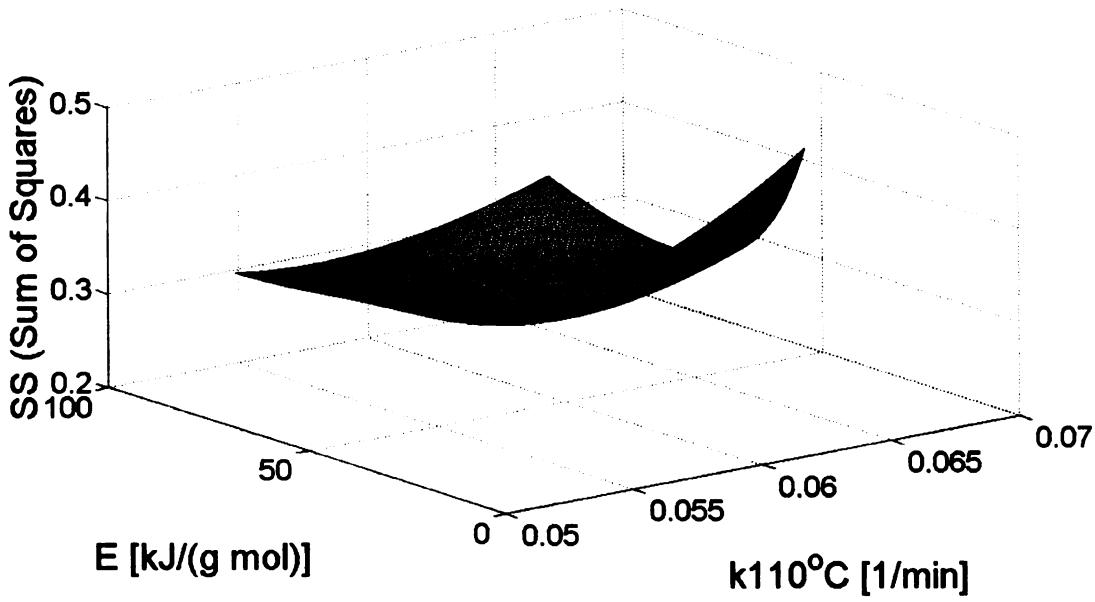


Figure 2.6 3-D Surface plot of Sum of Squares (SS)

2.5 Conclusions

The method described in this study will be useful in determining the change in nutraceuticals concentration and the prediction error for non-isothermal processes in low-/intermediate-moisture food during high-temperature processing, which can be a valuable tool for various food industries engaged in making functional foods. This paper presents three novel results:

1. Using both Comsol (finite-element) and Matlab (non-linear regression) for nonlinear regression;

2. This method is more powerful because it uses *k* and *Cp* as functions of temperature and takes into account the varying boundary condition during the lag time;

3. It gives CB and PB for nutrient retention, as well as the CI for the parameters.

For the processors, the sizes of CB and PB for nutrient retention are more important than size of CI of the parameters. The parameter estimation is the intermediate step to obtain the nutrient retention CB and PB.

2.6 Nomenclature

α	thermal diffusivity, m^2/s
ρ	density, kg/m^3
σ	standard error
ρ	correlation coefficient
a, b and c	thermal conductivity parameters (eq. (2))
a′, b′ and c′	specific heat parameters (eq. (3))
$\overline{C}$	mass-average anthocyanins concentration within a can, mg/mL
C_o	initial mass-average anthocyanin concentration, mg/mL
C_p	Specific heat, J/kg ^oC
CB	confidence band
CI	confidence interval
E_a	activation energy, J/g-mol
H	convective heat transfer coefficient, W/m^2K
k	thermal conductivity, W/m K
k_r	degradation rate constant at reference temperature T_r, min^{-1}
N	number of points for the trapezoidal rule
n	number of data
p	number of parameters
PB	prediction band
r	dimensionless radius
R_g	gas constant (J/g-mole K)
R	container radius, m

RMSE	root mean square error, g/g or °C
T	temperature (K)
T_i	initial temperature, K
T_r	reference temperature = 383 K
w	Gauss weights
Y	mass average retention of anthocyanins, fractional g/g.
z	dimensionless axial position, where z = 0 is at the half-height point of the can

2.7 References

Ahmed J, Shivhare US & Raghavan GSV. 2004. Thermal degradation kinetics of anthocyanin and visual colour of plum puree. European Food Research and Technology 218(6):525-528.

Alexandra Pazmino-Duran E, Monica Giusti M, Wrolstad RE & Gloria MBA. 2001. Anthocyanins from Oxalis triangularis as potential food colorants. Food Chemistry 75(2):211-216.

Calvi JP & Francis FJ. 1978. Stability of concord grape (v. labrusca) anthocyanins in model systems. Journal of Food Science 43(5):1448-1456.

Camire ME, Chaovanalikit A, Dougherty MP & Briggs J. 2002. Blueberry and Grape Anthocyanins as Breakfast Cereal Colorants. Journal of Food Science 67(1):438-441.

Camire ME, Dougherty MP & Briggs JL. 2007. Functionality of fruit powders in extruded corn breakfast cereals. Food Chemistry 101(2):765-770.

Cemeroglu B, Velioglu S & Isik S. 1994. Degradation Kinetics of Anthocyanins in Sour Cherry Juice and Concentrate. Journal of Food Science 59(6):1216-1218.

Dolan KD & Steffe JF. 1989. Back extrusion and simulation of viscosity development during starch gelatinization. Journal of Food Process Engineering 11(2):79-101.

Dolan KD, Yang L & Trampel CP. 2007. Nonlinear regression technique to estimate kinetic parameters and confidence intervals in unsteady-state conduction-heated foods. Journal of Food Engineering 80(2):581-593.

Kenneth J. Valentas RPS, Enrique Rotstein. 1997. Handbook of food engineering practice. CRC press.

Kirca A & Cemeroglu B. 2003. Degradation kinetics of anthocyanins in blood orange juice and concentrate. Food Chemistry 81(4):583-587.

Lai KPK. 2003. Modeling thermal and mechanical degradation of anthocyanins in extrusion processing. M.S. Thesis, Department of Biosystems and Agricultural Engineering. East Lansing: Michigan State University. p. 72-74.

Markakis P, Livingston GE & Fellers CR. 1957. Quantitative aspects of strawberry pigment degradation. Journal of Food Science 22(2):117-130.

Montgomery DP, EA; Vining, GG. . 2006. Introduction to linear regression analysis.,4th edition. ed.: John Wiley & sons, inc.

Nasri H, Simpson R, Bouzas J & Torres JA. 1993. Unsteady-state method to determine kinetic parameters for heat inactivation of quality factors: Conduction-heated foods. Journal of Food Engineering 19(3):291-301.

Rob van den Hout GMKvtR. 1999. Modelling of the inactivation kinetics of the trypsin inhibitors in soy flour. Journal of the Science of Food and Agriculture 79(1):63-70.

Sastry I, Tischer, rg. 1953. Behavior of the anthocyanin pigments in concord grapes during heat processing and storage.

Seber GAF, & Wild, C.J. , NY. . 1989. Nonlinear regression. NY.: John Wiley & Sons,.

Skrede G WR, Durst RW. 2000. Changes in Anthocyanins and Polyphenolics During Juice Processing of Highbush Blueberries (Vaccinium corymbosum L.). . J Food Sci 65(2):357-364.

St. S. Tanchev NY. 1974. Kinetics of Thermal Degradation of the Anthocyanins Delphinidin-3-rutinoside and Malvidin-3-glucoside. Food / Nahrung 18(8):747-752.

Wang H, Nair MG, Strasburg GM, Chang YC, Booren AM, Gray JI & DeWitt DL. 1999. Antioxidant and Antiinflammatory Activities of Anthocyanins and Their Aglycon, Cyanidin, from Tart Cherries. J. Nat. Prod. 62(2):294-296.

Welt BA, Teixeira, A.A., Balaban, M.O., Smerage, G.H., Hintinlang, D.E., & Smittle, B.J. 1997. Kinetic parameter estimation in conduction heating

foods subjected to dynamic thermal treatments. J. Food Sci. 62(3):529-534,538.

Youdim KA, McDonald J, Kalt W & Joseph JA. 2002. Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults. The Journal of Nutritional Biochemistry 13(5):282-288.

Zhang Z, Kou X, Fugal K & McLaughlin J. 2004. Comparison of HPLC Methods for Determination of Anthocyanins and Anthocyanidins in Bilberry Extracts. J. Agric. Food Chem. 52(4):688-691.

CHAPTER 3

Multi-parameter Estimation and Parameter Confidence Regions for Degradation of Anthocyanins in Grape Pomace

Abstract

Confidence intervals on parameters and predicted retention values can help design food processes that would improve food quality and safety. In this study, several models were used to predict the degradation of nutraceuticals in low-moisture foods processed at higher temperatures. Grape pomace with different moisture contents of 17, 34, and 42% (wb) was heated in 202x214 steel cans in a retort at 126.7°C for times ranging from 8 to 25 minutes. Temperature in canned grape pomace during conduction heating was predicted by comsol® using known quadratic models for thermal properties. Anthocyanin retention in retorted samples was measured using HPLC. Rate constant (k_{110^oC}), activation energy (E_a) and moisture content parameter b , initial anthocyanin concentration (C_o) and reference temperature (T_r) were estimated using nonlinear regression in matlab®. Confidence band and the prediction band were computed for the predicted Y (anthocyanin degradation). Inference regions and joint confidence regions for k_{110^oC} and E_a were estimated using the iterative methods for all the models. The activation energy E_a, rate of reaction k_{110^oC} and the moisture parameter b estimated using nonlinear regression, were 75.34 kJ/g mol, 0.058 min^{-1} and 1.87 $(MC_{wb})^{-1}$. The availability of the software and the statistical packages makes nonlinear regression accessible for all researchers. Other investigators can consider reporting confidence intervals and confidence regions using the methods presented, for maximizing use of limited data sets and improving quality of food.

3.1 Introduction

Kinetic models often involve two parameters, such as k_r-E_a in arrhenius model, D_r-z in microbial model and V_{max}-K_m in the Michaelis-Menten model. Usually these parameters are estimated using isothermal experiments. The procedure for estimating parameters from isothermal data is mathematically straightforward and well established. However, the commercial processes are usually dynamic, i.e. have time-varying temperatures or other variables, such as time-varying moisture during a drying process. Moreover, many commercial processes for low-and intermediate-moisture foods have more than 2 parameters, because there are multiple variables, such as time, temperature, moisture content, shear rate, pH, pressure, etc. Therefore, kinetic analysis based on isothermal 2-parameter models does not apply to many of these commercial high-temperature processes for low- and intermediate moisture foods.

Use of high temperature (>100 °C) in food processing can lead to the degradation of some nutraceuticals. Because low-moisture foods processed at high temperature are necessarily heated nonisothermally, one must use nonlinear regression techniques for kinetic analysis. As there are no standard procedures or solutions available for the nonlinear models it is often difficult to estimate the parameters. Hence iterative techniques are the only tools to find a solution. Fitting the nonlinear models has become easier and quicker with the access and advancement in computer software. However, computing the confidence intervals can be a challenge in nonlinear models as the behavior of

parameter estimates are complex functions of the formulation of the equation, the parameterization, design of experiment and the residual variance (Watts, 1994).

Modeling nutraceutical degration can save time and money spent on actual lab experiments. Transformation of the equation to linear form in parameters and fitting it using linear least squares is not always a valid approach for nonlinear models. The 2-step isothermal approach usually leads to larger confidence intervals than the 1-step nonisothermal approach (Wendie L. Claeys, 2001a). Parameters should be estimated using a nonlinear approach with the original rate equation if the original rate data have constant variance. Confidence band and the prediction band for the predicted Y (dependent variable) can be computed along with the confidence interval of the parameters (Dolan and others 2007), which can provide important information regarding the safe processing as well as retaining the quality of food.

Confidence intervals for the parameter should be reported at a certain probability. For example, confidence interval for activation energy of 150 kJ/mol having a standard error of s can be reported as $150 \pm 2s$ at 95% probability. Confidence interval associated with the parameter can help determine quality (nutrient retention) or safety (microbial retention) of the processed food. In absence of estimates of the confidence intervals, safety factors used to be based on the industry practices and experience. The extra processing time (safety factor) implied for the food safety without any scientific basis may have unfavorable effect on processed food. This may result in over-processing, which

leads to degradation in color, texture, flavor and nutritive value (Lenz and Lund, 1977) or under-processing, which is favorable for microbial growth.

Confidence interval for the parameters is narrower if the temperature dependence is directly attached to the rate equation and the rate equation is analysed over all the temperature ranges (Boekel, 1996). 90% joint confidence regions were constructed to estimate the statistical accuracy of the simultaneously estimated parameters for thermal inactivation of alkaline phosphatase and lactoperoxidase and for the denaturation of β-actoglobulin (Wendie L. Claeys, 2001a). Joint confidence regions were also reported for the formatin kinetics of hydroxymethylfurfural, lactulose and furosine in milk at 90% confidence (Wendie L. Claeys, 2001b). 90% joint confidence intervals were reported for the D_r and z model for inactivation kinetics of freeze dried α-amylase from Bacillus amyloliquefaciens (Saraiva and others 1996). Parameter confidence regions were reported for kinetic parameters for microbial death in unsteady state conduction heated canned foods and for the thiamine concentration using a nonlinear regression method (Dolan et al., 2007).

In the context of nonisothermal processing and nonlinear regression there is need for a standard and user-friendly procedure to (a) simultaneously estimate more than two kinetic parameters for food processes, (2) estimate error in the parameters (confidence intervals and confidence regions) and in the measured Y

(confidence bands and prediction bands), (3) construct joint confidence region for simultaneously estimated parameters.

3.2 Mathematical Model

3.2.1 Parameter estimation using nonlinear regression

The parameters involved in the Arrhenius equation were estimated using Matlab in combination with Comsol using the following command;

[beta, residual, Jacobian] = nlinfit(X, y, @fun, beta0)

'fun' is a function that accept X as input variable and it is of the form;

yhat = fun(beta,X)

In this study 'fun' is a function that computes temperature inside the can.

The command 'nlinfit' in matlab® returns the estimated parameters, the residuals and the Jacobian using the least squares, which minimized the following equation:

$$SS = \sum_{i} [(Y_{obs})_i - (\hat{Y}_{pred})_i]^2 \quad (1)$$

3.2.1.1 Moisture content model

The degradation of anthocyanins at different temperatures can be modeled as first-order reaction kinetics (Ahmed and others 2004; Cemeroglu and others 1994),

$$\frac{dC}{dt} = -kC, \tag{2}$$

The quantification of the effect of temperature on the reaction rate can be well expressed by the Arrhenius model,

$$k = k_r e^{\frac{-E_a}{R}\left(\frac{1}{T} - \frac{1}{T_r}\right)} \tag{3}$$

under dynamic temperature conditions the degradation of anthocyanins can be expressed by,

$$\left(\overline{C}/C_o\right)_{pred} = 2\iiint e^{-k_r \int_0^t e^{\frac{-E_a}{R}\left(\frac{1}{T(r,z,t)} - \frac{1}{T_r}\right)} dt} \, r\, dr dz \tag{4}$$

If moisture content of the product is included as a variable, assuming an exponential effect and no moisture difference at different locations within the can, the equation becomes,

$$\left(\overline{C}/C_o\right)_{pred} = 2\iint e^{-k_r \int_0^t e^{\left(\frac{-E_a}{R}\left(\frac{1}{T(r,z,t)} - \frac{1}{T_r}\right) + b(MC(t) - MC_r)\right)} dt} \, r\, dr dz$$

(5)

For the case where the can is sealed, moisture content does not change with time and can be moved out of the time integral:

$$\left(\overline{C}/C_o\right)_{pred} = 2\iint e^{-k_r e^{b(MC - MC_r)} \int_0^t e^{\frac{-E_a}{R}\left(\frac{1}{T(r,z,t)} - \frac{1}{T_r}\right)} dt} \, r\, dr dz$$

(6)

and the parameters k_r, E_a and b were estimated simultaneously using the nonlinear regression method. Residual plots were also checked for the absence of trends or correlations.

3.2.1.2 Confidence Intervals

Asymptotic confidence intervals on the parameter estimates and predicted Y (anthocyanin retention) were calculated using the two commands in Matlab,

for parameters: nlparci(beta, residual, Jacobian, α)

The command 'nlparci(beta,resid,J,alpha)' returns the confidence interval on the nonlinear least squares parameter estimates beta at the level of confidence determined by alpha. 95% confidence intervals for each parameter were calculated using nlparci.

for predicted Y value: nlpredci(fun, x, beta, residual, Jacobian, α, 'simopt')

3.2.1.3 Standard Error and Correlation Coefficient

Information about the Standard error σ_i of parameters was obtained from the variance-covariance matrix (Boekel, 1996). The square root of the corresponding diagonal of the symmetrical variance-covariance matrix provides the information about σ_i of the corresponding parameter.

$$\mathrm{cov}(a) = (X^T X)^{-1}(MSE) = \begin{pmatrix} \sigma^2_{k_r} & \sigma_{k_r E_a} & \sigma_{k_r b} \\ \sigma_{k_r E_a} & \sigma^2_{E_a} & \sigma_{E_a b} \\ \sigma_{k_r b} & \sigma_{E_a b} & \sigma^2_{b} \end{pmatrix} \quad (7)$$

where, **X** is the Jacobian

$$\mathbf{X} = \begin{pmatrix} \left(\frac{\partial Y_1}{\partial \beta_1}\right) & \cdots & \left(\frac{\partial Y_1}{\partial \beta_p}\right) \\ \vdots & \ddots & \vdots \\ \left(\frac{\partial Y_n}{\partial \beta_1}\right) & & \left(\frac{\partial Y_n}{\partial \beta_p}\right) \end{pmatrix} \qquad (8)$$

The correlation coefficient $\rho_{ij} = \sigma_{ij} / \left(\sigma_i \sigma_j\right)$ varies from -1.0 to 1.0. Higher values of $|\rho|$ indicates more difficulty in the parameter estimation process in nonlinear regression method.

3.2.1.4 Confidence region

The confidence region is a set of values for the two parameters. Approximate inference regions for the estimated parameters using the nonlinear regression were drawn per the following equation (Bates, 1988),

$$(\theta - \hat{\theta})^{\mathrm{T}} \hat{\mathrm{V}}_1^{\mathrm{T}} \hat{\mathrm{V}}_1 (\theta - \hat{\theta}) \leq p s^2 F(p, N-p; \alpha) \qquad (9)$$

where, the derivative matrix $\hat{\mathrm{V}} = \hat{\mathrm{Q}}_1 \hat{\mathrm{R}}_1$ and it is calculated at $\hat{\theta}$.

The boundary of the inference region as mentioned above is

$$\left\{\theta = \hat{\theta} + \sqrt{Ps^2 F(P, N-P;\alpha)}\ \hat{R}_1^{-1}\ d\ \ \|d\| = 1\right\} \tag{10}$$

This equation will provide an ellipsoid, which is an approximation of the true contour, and is used for computational efficiency, compared to the iterative method below.

For better accuracy, joint confidence regions were also plotted using the method of (Motulsky, 2004). This was done by obtaining the sum of squares of errors less than or equal to a constant value determined by the following equation,

$$SS_{all-fixed} = SS_{best-fit}\left(F\frac{P}{n-P}+1\right) \tag{11}$$

The contour of the joint confidence region was computed using the program in Matlab as follows: fix one of the parameters equals to best-fit value and allow the other parameter to vary until $SS \cong SS_{all\text{-}fixed}$. As the contour is oval shaped, there will be two values of the second parameter, one on each side of the oval and these two values are computed till they are almost equal (1 - 5% of each other). Then fix the parameter 1 at slightly lower value (~95% of the best-fit value) and again find the two values of the parameter 2. This routine completes the lower half of the contour. The upper half is computed in the same manner but using the increasing values of the fixed parameter.

For multi-parameter models, plotting the joint confidence regions is not possible (Bates, 1988). Hence, making pairs of the parameters while holding the other parameters constant will result in plots of the joint confidence regions.

3.3 Methods and Materials

3.3.1 Thermal treatment and degradation of anthocyanins

Retort simulation, extraction of anthocyanins and high performance liquid chromatography (HPLC) was adopted from (Mishra and others 2008). Briefly, grape pomace having moisture content of 42, 34 and 17% (wet basis) was separately heated in a steam retort at 126.7 °C for time ranging from 8 to 25 minutes. These heating experiments were done in triplicate. After heating, the grape pomace in each can was mixed for 40 seconds in a food grinder (Cuisinart® Mini Prep plus) to ensure sufficient mixing for uniform sampling from each can. Preliminary HPLC experiments showed that mixing for this time gave coefficient of variance for anthocyanin content at different locations in one can of < 10%. Retorted samples were then extracted for anthocyanins using the extraction solvent (Mishra et al., 2008) and then the solution was kept for further analysis. HPLC with a dual wavelength detector system was used to detect the individual anthocyanidin peaks. Anthocyanin concentration ($\mu g / ml$) was

computed by comparing the area under the all the five peaks (delphinidin, cyanidin, petunidin, peonidin, malvidin)
to the standard curve of Pelargonidin. Anthocyanidin reference standards delphinidin chloride, cyanidin chloride, peonidin chloride and malvidin chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA); petunidin chloride was purchased from Extrasynthèse (Genay Cedex, France)

Anthocyanidin standard curves were prepared by dissolving delphinidin chloride in methanol; cyanidin chloride was dissolved in 5% hydrochloric acid in 80% ethanol; malvidin chloride was dissolved in 95% ethanol; and petunidin chloride was dissolved in methanol acidified with 0.1% hydrochloric acid. All samples were dissolved at 1 mg / mL solvent. Appropriate dilutions were then made using the solvent recommended to generate a standard curve. The retention value of the anthocyanin was then calculated by dividing each concentration by the mean of the raw grape pomace anthocyanin contents.

3.4 Results

The anthocyanins retention for the grape pomace at different moisture content as measured using HPLC was as follows (Table 3.1);

Table 3.1: Heating time and measured anthocyanin retention for grape pomace at 17, 34 and 42% (wb) moisture content at 126.7 °C retort temperature.

Sample number	Heating time (min.)	Retention of anthocyanins	MC(wb)
1	0	1.117	0.42
2	0	0.855	0.42
3	0	0.855	0.42
4	0	0.883	0.42
5	0	1.029	0.42
6	0	0.984	0.17
7	0	1.007	0.17
8	0	1.110	0.34
9	0	1.159	0.34
10	8	0.837	0.42
11	8	0.733	0.42
12	8	0.804	0.42
13	10	0.688	0.42
14	10	0.684	0.42
15	10	0.686	0.42
16	12	0.698	0.42
17	12	0.744	0.42
18	12	0.714	0.42

Table 3.1 Con't

Sample number	Heating time (min.)	Retention of anthocyanins	MC(wb)
19	14	0.565	0.42
20	14	0.515	0.42
21	14	0.409	0.42
22	15	0.498	0.42
23	15	0.507	0.42
24	15	0.599	0.42
25	15	0.847	0.17
26	15	0.788	0.17
27	15	0.720	0.17
28	15	0.603	0.17
29	15	0.521	0.17
30	15	0.560	0.17
31	15	0.753	0.34
32	15	0.587	0.34
33	15	0.821	0.34
34	15	0.450	0.34
35	15	0.429	0.34
36	15	0.587	0.34
37	16	0.341	0.42
38	16	0.285	0.42
39	16	0.433	0.42
40	16	0.467	0.17
41	16	0.316	0.17
42	16	0.463	0.17

Table 3.1 Con't

Sample number	Heating time (min.)	Retention of anthocyanins	MC(wb)
43	17	0.301	0.42
44	17	0.309	0.42
45	17	0.305	0.42
46	19	0.212	0.42
47	19	0.241	0.42
48	19	0.227	0.42
49	21	0.234	0.42
50	21	0.188	0.42
51	21	0.249	0.42
52	23	0.218	0.42
53	23	0.222	0.42
54	23	0.213	0.42
55	25	0.170	0.42
56	25	0.155	0.42
57	25	0.145	0.42

The estimated kinetic parameters for the anthocyanin degradation obtained from the nonlinear regression technique are provided in Table 3.2. The root mean square error was found to be 0.109, which is ~11% of the total scale showing good fit as shown in fig. 3.1. The correlation coefficient among the parameters is shown in table 3.2. As processors would be more interested in the PB, a plot of PB was plotted along with the predicted Y and CI on the predicted Y (fig 3.1).

Table 3.2: Kinetic parameters for anthocyanin degradation in grape pomace at 17, 34 and 42% (wb) moisture content

Parameter	No. of Data	Parameter Estimates	Standard Error	Correlation coefficient (ref. temperature)	95% asymptotic confidence interval	RMSE
k_{110^oC}	57	0.058 min^{-1}	0.0043	$\rho_{k_r E_a}$ =-0.5 ρ_{b,E_a} =0.029 $\rho_{k_r,b}$ = 0.43 T_r =110°C	(0.049, 0.066)	0.109
E_a		75.34 kJ/g mol	26.5		(22.1, 128.5)	
B		1.87 $(MC_{wb})^{-1}$	0.57		(0.71, 3.02)	

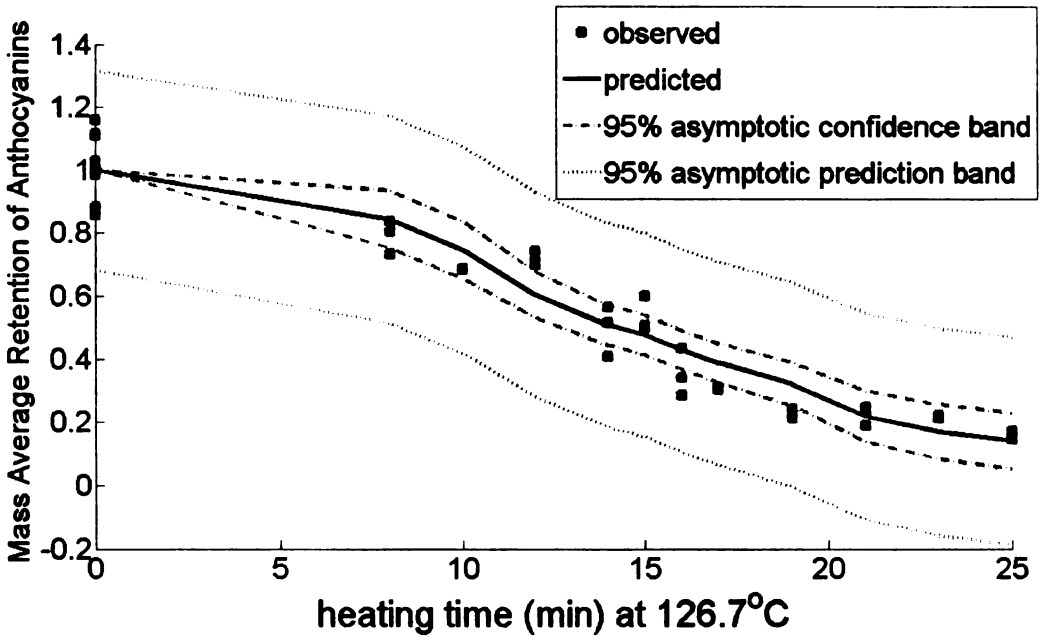


Figure 3.1: 95% asymptotic confidence band and 95% asymptotic prediction band for mass average retention of anthocyanins in grape pomace at 42% moisture content (wb) heated in 202 x 214 cans at retort temperature of 126.7 ^{o}C.

To check for the absence of trends or correlations between the parameters, a residual plot was constructed. The residuals followed a normal distribution and were found to be scattered around the center (Fig. 3.7) This plot shows a good fit of the modified Arrhenius model to accommodate the change in moisture content to the degradation of anthocyanins in grape pomace.

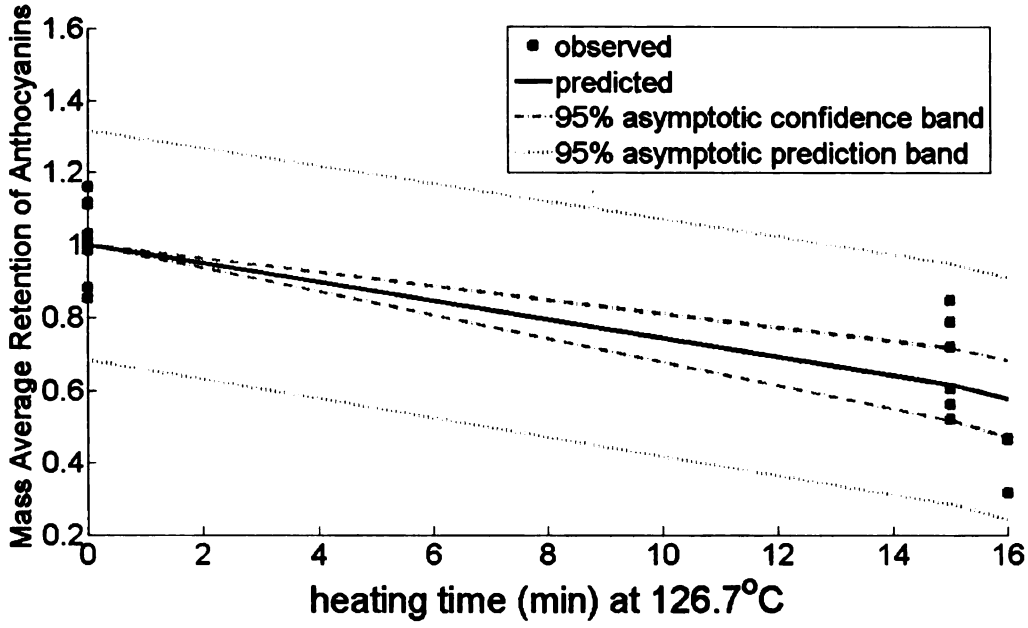


Figure 3.2: 95% asymptotic confidence band and 95% asymptotic prediction band for mass average retention of anthocyanins in grape pomace at 17% moisture content (wb) heated in 202 x 214 cans at retort temperature of 126.7 ^{o}C.

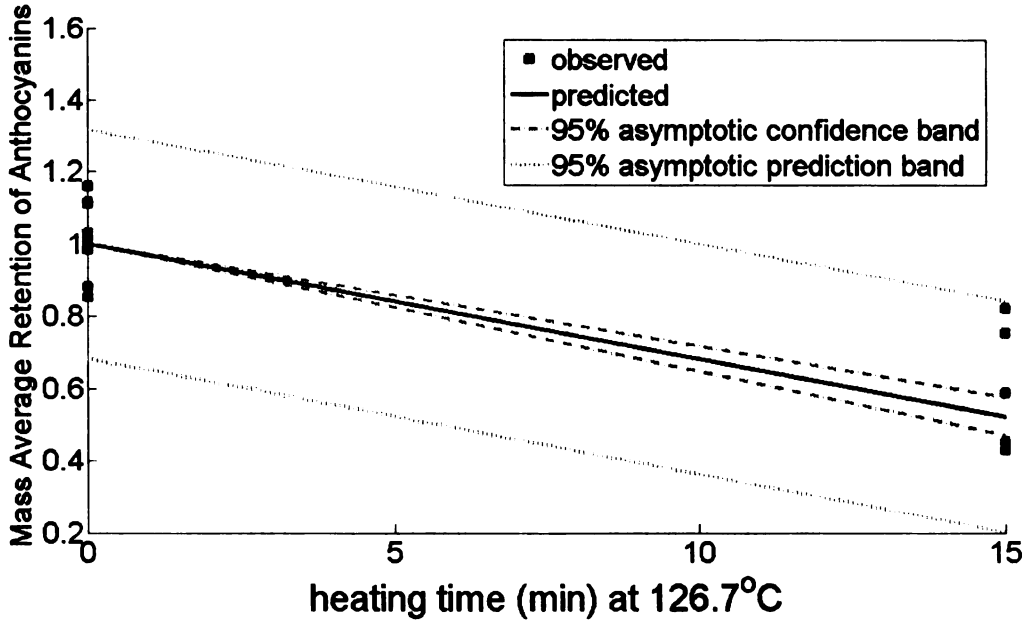


Figure 3.3: 95% asymptotic confidence band and 95% asymptotic prediction band for mass average retention of anthocyanins in grape pomace at 34% moisture content (wb) heated in 202 x 214 cans at retort temperature of 126.7 oC.

Kinetic parameters for the anthocyanin degradation obtained from this study are comparable to the values for anthocyanin degradation in extruded flour mixed with grape pomace (Lai, 2003): The activation energy was 76.0 kJ/g-mol, the rate of reaction (k_{80}) was 0.049 min^{-1} and the value of b was 5.28 MC_{wb}^{-1}whereas in this study the value of activation energy was found to be 75.34 kJ/g-mol, rate of reaction k_{80} was 0.011 min^{-1} and the value of b was 1.87 MC_{wb}^{-1}. As expected, the degradation rate k_{80} and the activation energy in the intermediate-moisture food was nearly same in both the studies.

95% joint confidence region was plotted as an ellipse in Figure 3.4. Moreover, the true 95% joint confidence region was also plotted on the same plot using the Motulsky method (Motulsky, 2004). The sum of squares around the contour was also calculated in both the cases and it was found that the SS was constant along the contour in case of Motulsky while the SS was not constant along the approximate elliptical contour, which shows that approximate CRs may have significant errors.

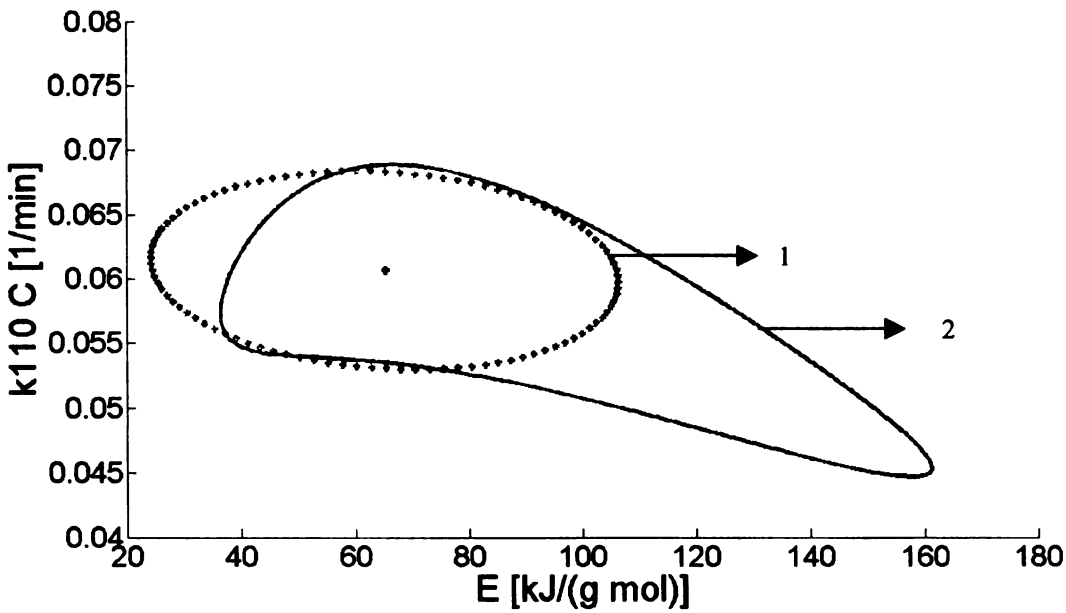


Figure 3.4: 95% joint confidence region using (1) equation (9) and (2) Motulsky method for mass average retention of anthocyanins in grape pomace 42% moisture content (wb) with parameters having correlation coefficient of –0.5.

The 3-D plot of sum of squares was constructed using Matlab for the kinetic parameters E_a and k_{110} for the grape pomace at 42% moisture content (wb). These plots provide information about the nature of standard error and confidence intervals, and the relative ease of convergence of the nonlinear

regression. Fig. 3.5 shows that the surface is shallower along the E-axis than along the $k_{110°C}$ axis, causing the standard errors and confidence intervals for E_a to be proportionately larger than that for $k_{110°C}$, consistent with the CI results in Table3.4. Better convergence can be obtained if the curve shows steeper change along the E_a axis. This plot was compared with the SS plot constructed for Welt's data for microbial death of *Bacillus stearothermophilus* (Welt, 1997). Fig. 3.7 shows a good convergence on both the parameters E_a as well as k_{110}. Because there is a steeper surface down towards the minimum SS, this visual check shows that the microbial death data of Welt et al. fit the Arrhenius model (equ. 3) better than our anthocyanin degradation data. The shallower surface might be improved by collecting more data at different moisture content and at different times. Welt et al.'s confidence intervals for E were proportionately smaller than ours, a result that could be deduced by examining these SS surface plots.

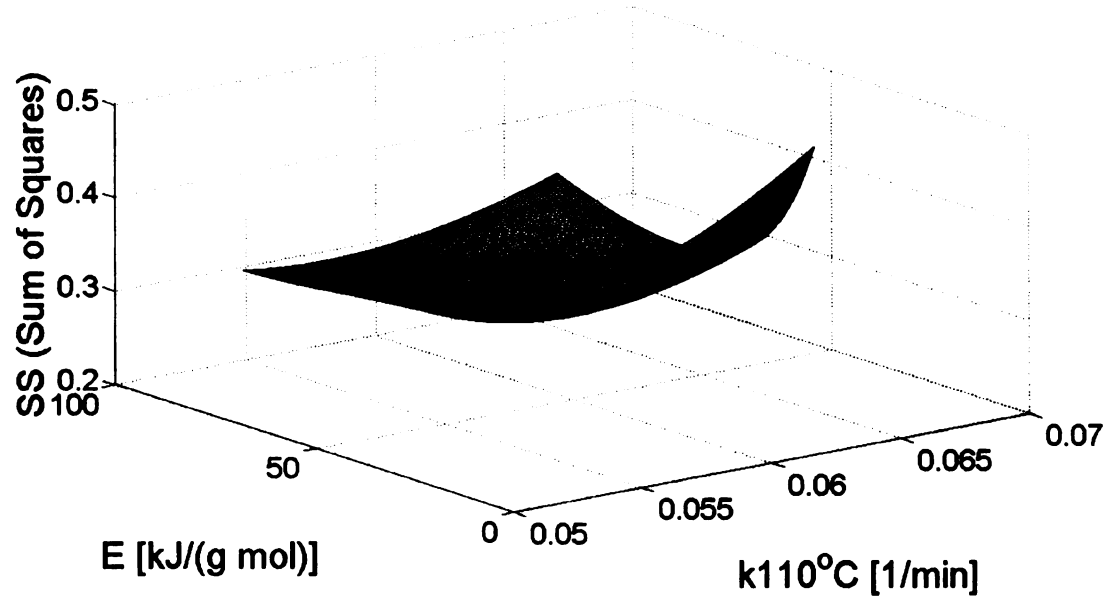


Figure 3.5: 3-D Surface plot of Sum of Squares (SS)

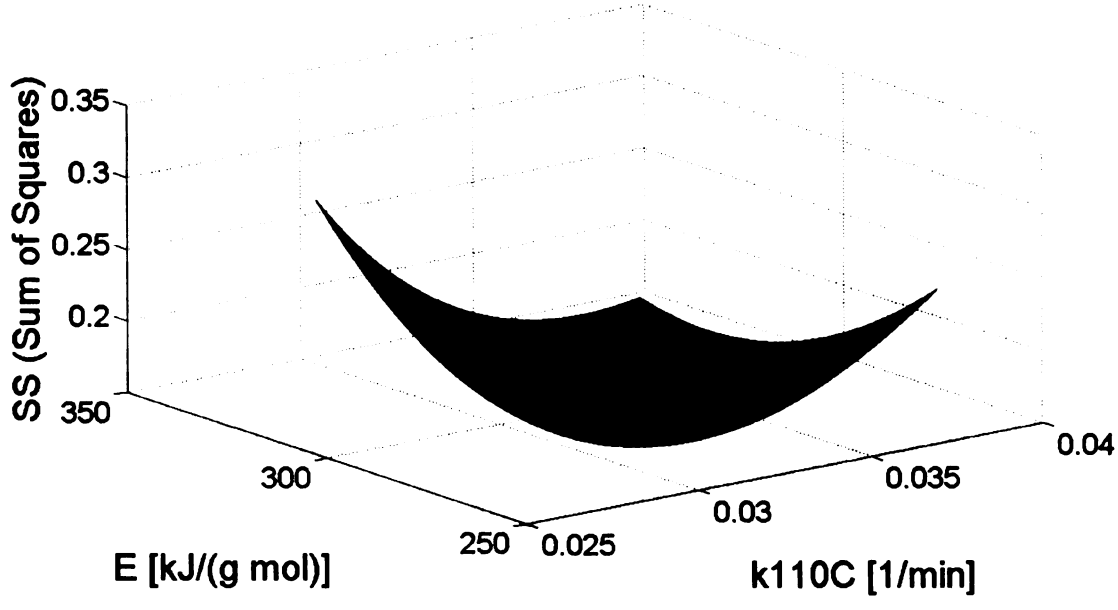


Figure 3.6: 3-D Surface plot of Sum of Squares (SS) for Welt's Data (Welt, 1997)

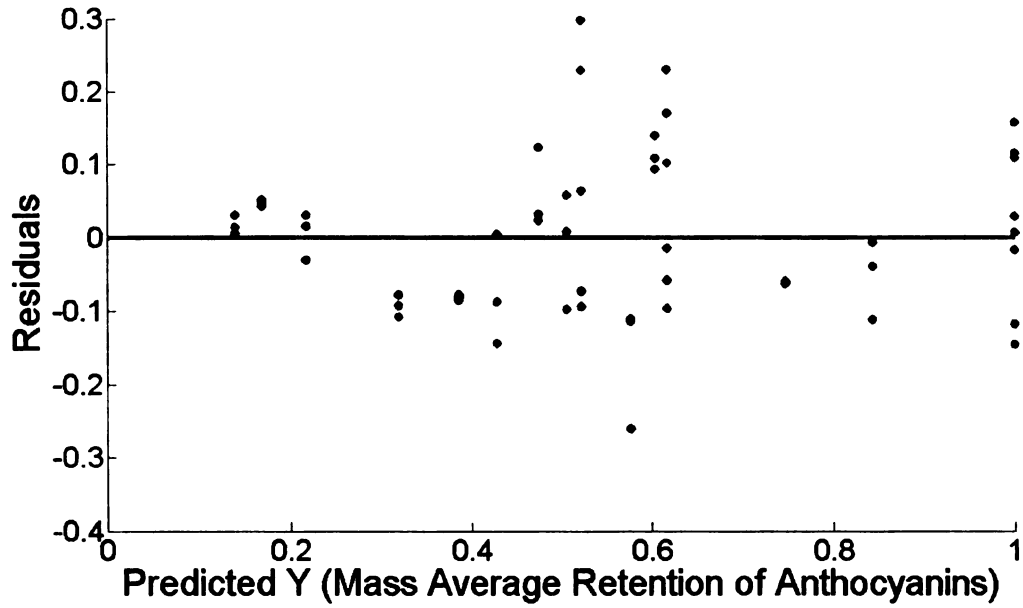


Figure 3.7: Residual plot of the mass average Retention of anthocyanins for Grape pomace at 17, 34 and 42% moisture content (wb) heated at retort temperature of 126.7 ^{o}C

3.5 Conclusion

The multi parameter approach to model nutraceutical degradation is a good method, as it incorporates the challenges faced in the processing industry, such as the simultaneous effect of temperature and moisture content, and potentially could describe the effect of other factors such as pH, pressure and viscosity. Confidence intervals and the joint confidence regions provide useful information about the nature of the parameters and their correlation. The net effect of these parameters is shown by plotting confidence bands (for the regression line) and prediction bands (for individual data). This paper shows a novel method to

estimate the parameters using nonlinear method for low-and intermediate-moisture food processed at high-temperature. Determining the confidence interval on parameters for the change in nutraceuticals concentration for non-isothermal heating will be a valuable tool for researchers and various food industries designing functional foods processes. By obtaining more information from limited data sets, these methods can help reduce experimental effort and cost, both major concerns for academia and industry. For researchers without access to Matlab, all statistical calculations can be done in Excel. For researchers without a Comsol or similar software, temperatures during conduction heat transfer can be approximated using the analytical solution with Excel (Dolan, 2007).

3.6 nomenclatures

$\left(\bar{C}/C_o\right)$	anthocyanin retention, where $0 \le \left(\bar{C}/C_o\right) \le 1$)
C_o	initial mass-average anthocyanins concentration
σ	standard error
p	number of parameters
ρ	correlation coefficient
E_a	activation energy, J/g-mol
Kk	rate constant min^{-1}
K	thermal conductivity, W/m K
k_r	rate constant at reference temperature T_r, min^{-1}
$\bar{C}$	mass-average anthocyanins concentration
n	number of data
R	gas constant (J/g-mole K)
r	dimensionless radius
R	container radius, m
RMSE	root mean-square error = $\sqrt{\Sigma(Y_i - \hat{Y}_i)^2/(n-p)}$
T	temperature (K)
T_i	initial temperature, K
T_r	reference temperature, K
z	dimensionless axial position
SS	sum of squares

$SS_{best\text{-}fit}$	minimum sum-of-squares of errors, i.e. sum-of-squares when nonlinear regression fits the curve
$SS_{all\text{-}fixed}$	target value for sum-of-squares to compute parameter joint confidence regions
p	number of parameters
$\hat{\theta}$	parameter estimates
F	critical value of the F distribution for a given confidence level and degrees of freedom. For example, in Excel, finv(95% confidence, n, n-p) = finv(0.05,2,28) = 3.34. In matlab, the identical expression is finv(0.95,2,28) = 3.34.

3.7 References

Ahmed J, Shivhare US & Raghavan GSV. 2004. Thermal degradation kinetics of anthocyanin and visual colour of plum puree. European Food Research and Technology 218(6):525-528.

Bates DM, & Watts, D.G. . 1988. Nonlinear regression analysis and its applications. New York: Wiley.

Boekel MAJS. 1996. Statistical Aspects of Kinetic Modeling for Food Science Problems. Journal of Food Science 61(3):477-486.

Cemeroglu B, Velioglu S & Isik S. 1994. Degradation Kinetics of Anthocyanins in Sour Cherry Juice and Concentrate. Journal of Food Science 59(6):1216-1218.

Dolan KD, Yang L & Trampel CP. 2007. Nonlinear regression technique to estimate kinetic parameters and confidence intervals in unsteady-state conduction-heated foods. Journal of Food Engineering 80(2):581-593.

Lai KPK. 2003. Modeling thermal and mechanical degradation of anthocyanins in extrusion processing. Department of Agricultural Engineering. East Lansing: Michigan State University. p. 72-74.

Lenz MK & Lund DB. 1977. The lethality-fourier number method: confidence intervals for calculated lethality and mass-average retention of conduction-heating, canned foods. Journal of Food Science 42(4):1002-1007.

Mishra DK, Dolan KD & Yang L. 2008. Confidence Intervals for Modeling Anthocyanin Retention in Grape Pomace during Nonisothermal Heating. Journal of Food Science 73(1):E9-E15.

Motulsky HJ, & Christopoulos, A. . 2004. Fitting models to biological data using linear and nonlinear regression. A practical guide to curve fitting. New York Oxford University Press.

Saraiva J, Oliveira JC, Hendrickx M, Oliveira FAR & Tobback P. 1996. Analysis of the Inactivation Kinetics of Freeze-dried alpha-Amylase from Bacillus

amyloliquefaciens at Different Moisture Contents. Lebensmittel-Wissenschaft und-Technologie 29:260-266.

Watts DG. 1994. Estimating parameters in nonlinear rate equation. The Canadian journal of chemical engineering 72.

Welt BA, Teixeira, A.A., Balaban, M.O., Smerage, G.H., Hintinlang, D.E., & Smittle, B.J. 1997. Kinetic parameter estimation in conduction heating foods subjected to dynamic thermal treatments. J. Food Sci. 62(3):529-534,538.

Wendie L. Claeys LRL, Ann M. Van Loey and Marc E. Hendrickx 2001a. Inactivation kinetics of alkaline phosphatase and lactoperoxidase, and denaturation kinetics of [beta]-lactoglobulin in raw milk under isothermal and dynamic temperature conditions. Journal of Dairy Research 68 95-107.

Wendie L. Claeys LRL, Marc E. Hendrickx 2001b. Formation kinetics of hydroxymethylfurfural, lactulose and furosine in milk heated under isothermal and non-isothermal conditions. Journal of Dairy Research 68:287-301.

CHAPTER 4

Bootstrap confidence interval for the kinetic parameters for degradation of anthocyanins in grape pomace

Abstract

Due to the growing interest in nutraceuticals and their health benefits, it is important to develop tools for modeling degradation of nutraceuticals in low-moisture and high temperature heated foods. The objective of this study was to estimate the thermal and kinetic parameters for the degradation of anthocyanins in grape pomace and to calculate the bootstrap confidence interval. Thermal and kinetic parameters for unsteady-state conduction-heated foods (grape pomace) were estimated using nonlinear regression techniques. Rate constant (k_{110^oC}) and activation energy (E_a) for the degradation of anthocyanins in grape pomace were estimated and bootstrap confidence intervals were calculated and compared to the 95% asymptotic confidence intervals. Grape pomace at 42% moisture content (wb) was heated in a steam retort at 126.7 °C in steel cans (radius 0.027m, and height 0.073m). Anthocyanin degradation was measured by high performance liquid chromatography. The degradation values were used to estimate kinetic parameters, which were k_{110^oC} =0.06 min^{-1} and E_a=65.3 kJ/mol. Asymptotic confidence intervals for k_{110^oC} and E_a were (0.055, 0.067) and (32.6, 97.9), respectively. Bootstrap 95% confidence intervals for k_{110^oC} and E_a were (0.055, 0.066) and (50.9, 100.82), respectively. Bootstrap confidence band and prediction bands for anthocyanin retention were smaller than asymptotic confidence and prediction bands, respectively. The smaller width of the bootstrap bands, which are considered more accurate than asymptotic bands, allows more accurate process design and cost-savings, potentially leading to higher-quality nutraceutical products.

4.1 Introduction

Estimation of parameters for isothermal processes, such as degradation of nutraceutical compounds in foods with high moisture content, is well established. Isothermal experiments can be performed with high-moisture food samples as the temperature gradient is very small and isothermal heating has very short lag times. The rate of reaction and the activation energy for the degradation of nutraceuticals can be computed by taking logarithm of the well-known Arrhenius equation as the process follows first-order reaction kinetics. Conducting isothermal experiments for the low- and intermediate-moisture foods is challenging because of the large temperature gradients within the sample and long lag times. Hence, nonisothermal experimentation is required for the low moisture foods like extruded products, breads, jam and jelly and vegetable pastes. For nonisothermal processes, kinetic parameters can be estimated by nonlinear regression methods using the least square methods.

Statistical method is important tool to estimate the accuracy of estimated parameter. However, it is not widely used in modeling for nutraceutical retention. Some researchers have proposed methods to calculate the asymptotic confidence interval and the joint confidence region to get good estimates of the error and correlation on estimated parameters (Dolan and others 2007; Bates, 1988; Wendie L. Claeys, 2001). The Jackknife method for the estimation of the experimental error on parameters was used for the kinetic model for thiamine destruction in pea puree (Nasri and others 1993). However, confidence intervals on predicted Y (microbial retention) and confidence intervals of the parameters

were not reported. Monte Carlo method was used to estimates the confidence intervals for mass average retention of conduction-heated canned foods (Lenz and Lund, 1977), but the confidence intervals on predicted Y were not reported. novel method has been proposed (Dolan et al., 2007) to calculate the confidence and prediction band on the predicted Y. In the literature surveyed, there were very few confidence intervals reported for parameters and none for predicted Y, which is the most important variable for processors.

Some researchers have applied the method of bootstrapping to get the confidnce intervals on the estimated parameters. However, no study showed computation of confidence intervals using bootstrap for degradation of nutracuticals in food materials. The purpose of this study is to provide a tool to the future researchers who can apply this method to report the confidence intervls on the parameters thereby providing safety and quality to the processed food. In the context of providing realistic estimate of error on the parameters involed in nonisothermal processing, the objectives of this study were to (1) compute bootstrap confidence intervals of kinetic parameters, (2) compute bootstrap confidence bands and bootstrap, (3) prediction bands for predicted anthocyanin retention, (4) compare bootstrap confidence and prediction values to their asymptotic counterparts.

4.2 Mathematical Model and Statistical Methods

4.2.1 Kinetic Parameter Estimation

A detailed discussion about the nonlinear regression technique can be found in (Mishra and others 2008). The parameter estimation process is described briefly in this paper. Thermal degradation of anthocyanins has been shown to follow first-order reaction kinetics (Ahmed and others 2004; Cemeroglu and others 1994),

$$\frac{dC}{dt} = -kC \tag{1}$$

The reaction rate can be quantified by the Arrhenius model;

$$k = k_r e^{\frac{-E_a}{R}\left(\frac{1}{T}-\frac{1}{T_r}\right)} \tag{2}$$

Under dynamic temperature conditions, retention of anthocyanins can be expressed by,

$$\left(\overline{C}/C_o\right)_{pred} = 2\iint e^{-k_r\int_0^t e^{\frac{-E_a}{R}\left(\frac{1}{T(r,z,t)}-\frac{1}{T_r}\right)}dt}\, r\, dr\, dz \tag{3}$$

where variables r and z were normalized; hence, the limits of both integrals were from 0 to 1. The kinetic parameters E_a and k_r were estimated using matlab® in combination with comsol® using the following command.

[beta, r, J] = nlinfit(X, y, @fun, beta0)

The command 'nlinfit' in matlab® returns the estimated parameters (beta), the residuals (r) and the Jacobian (J) using the least squares, which minimized the following equation to get the best sum of squares for the estimated parameters.

$$SS = \sum_i [(Y_{obs})_i - (\hat{Y}_{pred})_i]^2 \qquad (4)$$

Parameters k_r and E_a were estimated simultaneously using the nonlinear regression method. Residual plots were also checked for the absence of trends or correlations.

4.2.2 Overview of Bootstrap Method

The bootstrap method was found to be a better tool than the jackknife method, which was proved to be a linear approximation of the bootstrap method (Efron, 1979). If the distribution from which the samples are drawn is known and if the function is sufficiently tractable, the standard errors and hence the confidence intervals (CIs) can be constructed (Felsenstein, 1985). Bootstrap

procedure is most useful when the distribution of the sample is not known. When CIs for the parameters or retention are reported, typically they are asymptotic, because these are computationally efficient. Monte Carlo methods such as bootstrap, are known to produce more realistic CIs. Now that software is available for all most researchers, we can take advantage of these powerful statistical techniques to improve quality and safety of foods.

Bootstrap method, as it was found in the literature, follows the following procedure:

Step 1: For the data points $x_1, x_2, \ldots, x_n$, an estimate of parameter t can be obtained using a method T of statistical estimation (Felsenstein, 1985),

$$t = T\left(x_1, x_2, \ldots, x_n\right)$$

for example, T can be nlinfit in Matlab which is based on gauss-newton method of nonlinear regression.

Step 2: The bootstrap method allows resampling of the data to construct a fictional set of data. Each of these data sets is constructed by sampling n points with replacement from the x_n data. These fictional data sets consist of $x_1^*, x_2^*, \ldots, x_n^*$ points, where each x_i^* is drawn at random from the original data set. Then, t is again estimated for the new data set as,

$$t^* = T\left(x_1^*, x_2^*, \ldots, x_n^*\right)$$

Step 3: The sampling process i.e. step 2 is repeated many times to get a set of values of the estimated t^*. The distribution of this estimate approximates the distribution of the actual estimate t.

Step 4: Confidence limit on the parameter is calculated based on the upper or lower percentiles of the observed t^* values.

4.2.3 Application of Bootstrap to Kinetic Model

For 42 retention values of anthocyanins, predicted Y and time-temperature (tT) history i.e. $Y_1t_1T_1, Y_2t_2T_2, ..., Y_{42}t_{42}T_{42}$ were used to get an estimate of $\mathrm{E_a}$ and k_r;

$$[E_a, k_r] = \mathrm{nlinfit}(Y_1t_1T_1, Y_2t_2T_2, ..., Y_{42}t_{42}T_{42}) \quad (5)$$

Fictional data set was constructed by random sampling with replacement to get $\left(Y_1^*t_1^*T_1^*, Y_2^*t_2^*T_2^*, ..., Y_{42}^*t_{42}^*T_{42}^*\right)$. As the sampling was with replacement, there were chances that one data point occurred more than once, and that one data point did not occur at all. These new data sets were used to estimate the new set of parameters,

$$\left[E_a^*, k_r^*\right] = \mathrm{nlinfit}\left(Y_1^*t_1^*T_1^*, Y_2^*t_2^*T_2^*, ..., Y_{42}^*t_{42}^*T_{42}^*\right) \quad (6)$$

Random sampling process was repeated many times to get a set of values of the estimated $\left[E_a^*, k_r^*\right]$.

4.2.4 Bootstrap Confidence Interval on Parameters

The confidence limit on parameter was calculated based on the upper and lower 95 percentiles of the observed $\left[E_a^*, k_r^*\right]$ values. For example, the confidence interval on E_a for the $100.(1-\alpha)$ percentile can be obtained by,

$$\left(E_{a_{lo}}, E_{a_{up}}\right) = \left(\hat{E}_a^{*(\alpha/2)}, \hat{E}_a^{*(1-\alpha/2)}\right) \quad (7)$$

4.2.5 Bootstrap Confidence Interval on Predicted Y

The bootstrap confidence band on predicted Y was calculated using "nlpredci" in Matlab and bootstrap prediction band was calculated using the equation (Montgomery, 2006),

$$\text{prediction width}_i = \sqrt{(\text{confidence width}_i)^2 + (t_{\alpha/2,n-p} * RMSE)^2} \quad (8)$$

4.3 Methods and Materials

4.3.1 Experimentation

Analytical method to determine the anthocyanin content was adopted from (Mishra et al., 2008).In short, grape pomace (42% moisture content (wb)) was retorted in steel cans (radius 0.027m, and height 0.073m) in a rotary steam retort at 126.7 °C. The time was varied from 8 to 25 minutes to get the different level of degradation of anthocyanins in grape pomace. Extraction solvent (50 ml of methanol + 33 ml of water + 17 ml of 37% HCL) was used to extract the anthocyanins from the raw and retorted grape pomace. The quantification of anthocyanins was done by hydrolyzing the anthocyanins to anthocyanidins and was injected in HPLC (High performance Liquid Chromatography) system and the dual wavelength detector read the absorbance reading. The area under the peaks of chromatograms was used to calculate the anthocyanin content in the raw and retorted samples.

4.4 Results

Data used in this study was adopted from (Mishra et al., 2008). The heating time and retention of anthocyanins are provided in table 4.1.

Table 4.1: Retention $\left(\overline{C}/C_o\right)$ value of anthocyanins for grape pomace at 42% MC(wb) as measured from HPLC

Sample number	Heating time (min.)	Retention of anthocyanins
1	0	1.117
2	0	0.855
3	0	0.855
4	0	0.883
5	0	1.029
6	0	0.984
7	0	1.007
8	0	1.110
9	0	1.159
10	8	0.837
11	8	0.733
12	8	0.804
13	10	0.688
14	10	0.684
15	10	0.686
16	12	0.698
17	12	0.744
18	12	0.714
19	14	0.565
20	14	0.515
21	14	0.409
22	15	0.498
23	15	0.507
24	15	0.599
25	16	0.341
26	16	0.285
27	16	0.433
28	17	0.301
29	17	0.309
30	17	0.305
31	19	0.212

Table 4.1 Con't

Sample number	Heating time (min.)	Retention of anthocyanins
32	19	0.241
33	19	0.227
34	21	0.234
35	21	0.188
36	21	0.249
37	23	0.218
38	23	0.222
39	23	0.213
40	25	0.170
41	25	0.155
42	25	0.145

4.4.1 Kinetic Parameter Estimation

Kinetic parameters (table 4.2) from the nonlinear regression routine were obtained using the least square method. There were 42 data points, and the repeated converged value of the rate of reaction was 0.0606 min^{-1}, whereas the activation energy was found to be 65.32 kJ/gmol. Correlation coefficient ρ_{k,E_a} was –0.12. The root mean squared error was 0.084, which is approximately 9% of the total scale of 1.0, shows a good fit. 95% bootstrap confidence interval for k_{110^oC} was very close to the 95% asymptotic confidence interval but the bootstrap confidence interval on E_a was tighter than the asymptotic confidence interval for 95% confidenc.

Table 4.2: Kinetic parameters for anthocyanin degradation in grape pomace at 42% (wb) moisture content

Parameter	No. of Data	Parameter Estimates	Standard Error	95% Bootstrap confidence interval	95% asymptotic confidence interval	RMSE
k_{110^oC}	42	0.0606 min^{-1}	0.003	(0.053, 0.066)	(0.055, .067)	0.084
E_a		65.32 kJ/g mol	16.15	(49.08, 104.93)	(32.7, 97.9)	

4.4.2 Bootstrap Confidence Interval

The bootstrap confidence interval was calculated with random sampling of the original data set. Table 4.3 shows the confidence intervals at different number of time of bootstrapping. From these results it was concluded that 1000 bootstrap (Efron, 1987) was sufficient to get the confidence interval on the parameters.

Table 4.3 Bootstrap confidence interval at 95% on E_a and k_r

Number of bootstraps	Confidence interval E_a	Confidence Interval kr
10	(55.16, 82.73)	(0.0572, 0.0630)
100	(50.65, 115.92)	(0.0522, 0.0652)
200	(50.20, 107.10)	(0.0531, 0.0658)
500	(49.89, 106.54)	(0.0533, 0.0660)
1000	(49.08, 104.93)	(0.0531, 0.0660)
2000	(49.60, 106.28)	(0.0533, 0.0658)
5000	(49.31, 106.11)	(0.0532, 0.0659)

Figure 4.1 shows 95% bootstrap confidence band, 95% bootstrap prediction band, 95% asymptotic confidence band and 95% prediction band for mass average retention of anthocyanins. Correlation coefficient was –0.12. Many data fall outside the confidence band, as confidence band (CB) is the region where 95% of the regression lines are expected to be. The prediction band (PB) is the region where 95% of the data are expected to be. If more data were collected, we expect ~5% of all the data would fall outside the PB.

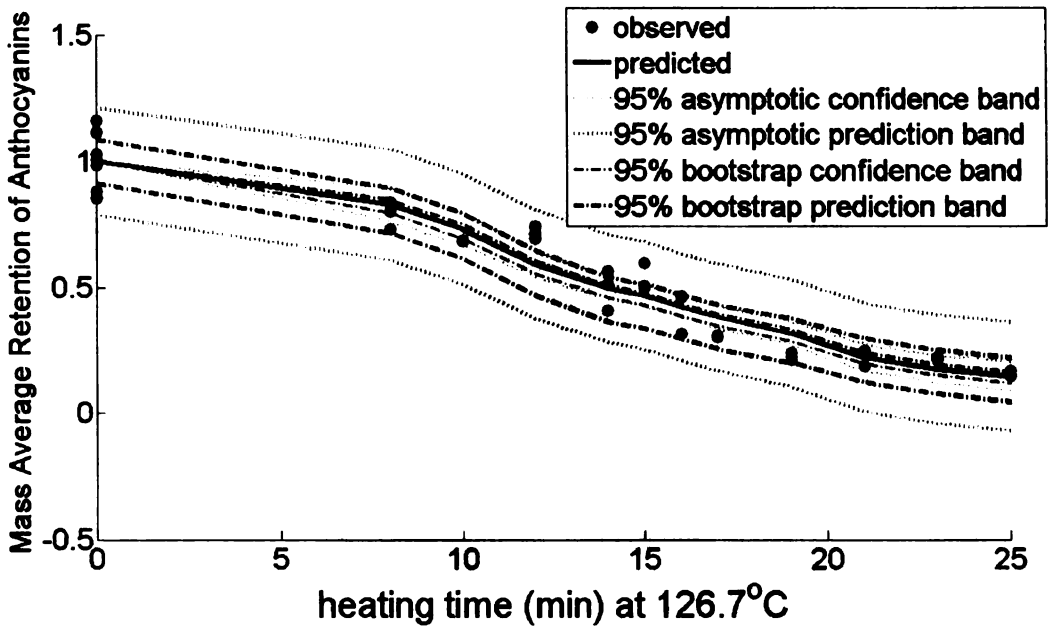


Figure 4.1: 95% bootstrap confidence band, 95% bootstrap prediction band, 95% asymptotic confidence band and 95% asymptotic prediction band for mass average retention of anthocyanins in grape pomace heated in 202 x 214 cans at retort temperature of 126.7 °C.

The confidence interval for the bootstrap is tighter in case of E_a whereas it was nearly same for the k_r as it has been shown in table 4.2. Confidence band for the predicted Y is very small for the bootstrap as compared to the asymptotic (fig.

1). Prediction band also shows the same trend for the bootstrap i.e bootstrap prediction band is much smaller than the asymptotic prediction band.

The normal bivariate distribution (figure 4.2) for the 5000 bootstrap parameters was plotted as a 3-D plot in Matlab. The contours of the confidence level of 90 and 95% were constructed out of the plotted distribution.

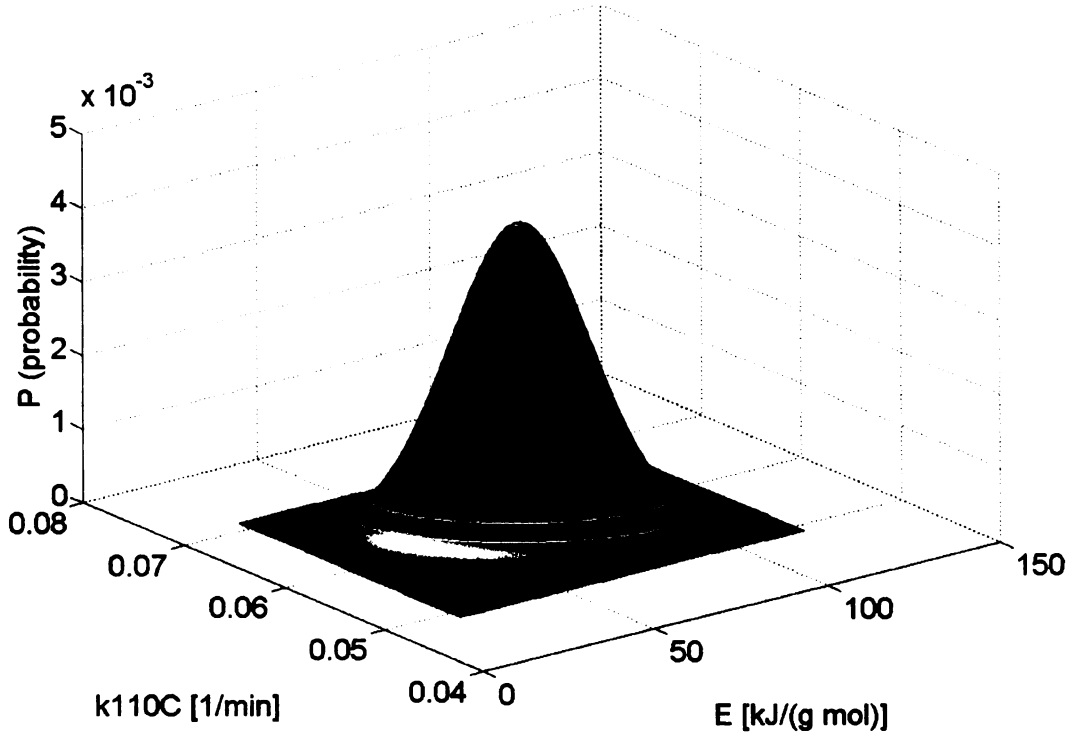


Figure 4.2 3-D plot of the bivariate normal distribution and 90 & 95% contours of the confidence level

Plot of 1000 bootstrap estimated parameters is shown in fig. 4.3. Joint confidence region was plotted on the same plot to compare confidence regions at 90 & 95% confidence level. 90% confidence level has the bigger region as it was expected, which covers 90% of the bootstrap estimated parameters.

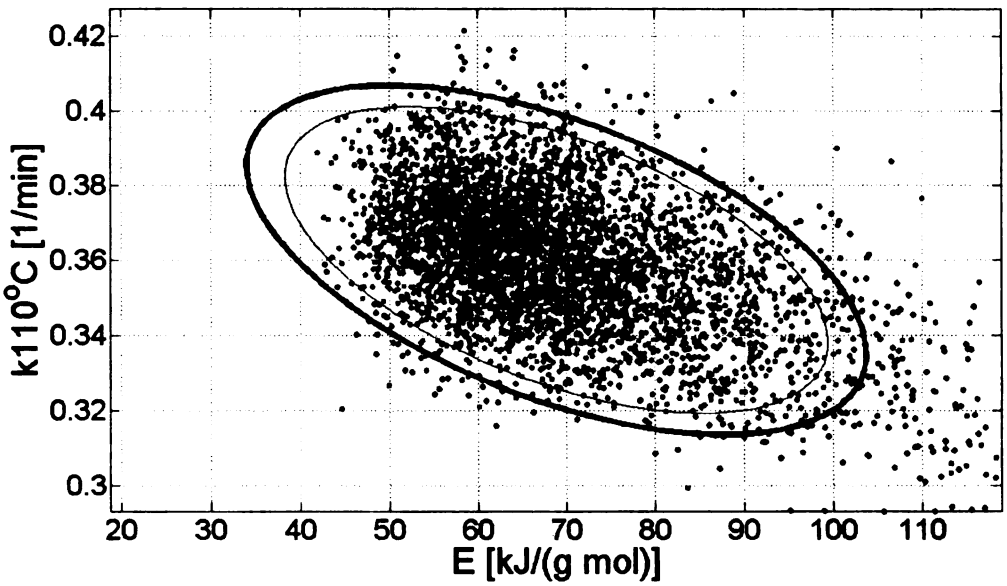


Figure 4.3 scatter plot of all the bootstrap estimated parameters and confidence regions at 90 & 95% confidence level (1,000 simulated points)

4.5 Conclusions

The bootstrap approach to determine the confidence interval on parameters for the change in nutraceuticals concentration for non-isothermal processes in low-/intermediate-moisture and high-temperature food during processing will be a valuable tool for researchers and various food industries engaged in making functional foods. Bootsrtap confidence interval is more advantageous if no other method of calculating the confidence interval is present. The bootstrap helps and shows the asymptoic confidence interval. This study was done with a hope that future researchers will use these methods to report the error estimates and confidence intervals, which will help processors to enhance the design of the processing system to have safe and quality food.

4.6 Nomenclatures

α	level of significance
ρ	density, kg/m^3
σ	standard error
ρ	correlation coefficient
$\overline{C}$	mass-average anthocyanins concentration within a can
C_O	initial mass-average anthocyanin concentration
C_p	Specific heat, J/kg °C
E_a	activation energy, J/g-mol
k	rate constant min^{-1}
k	thermal conductivity, W/m K
k_r	rate constant at reference temperature T_r, min^{-1}
n	number of data
p	number of parameters
r	dimensionless radius
R_g	gas constant (J/g-mole K)
R	container radius, m
$RMSE$	root mean square error, g/g or °C
t	Time
$t_{\alpha/2,n-p}$	t-value from T-test
T	temperature (K)
T_i	initial temperature, K
T_r	reference temperature = 383 K

w	Gauss weights
X	time-temperature history (independent variable)
Y	mass average retention of anthocyanins, fractional g/g.
z	dimensionless axial position, where z = 0 is at the half-height point of the can

4.7 References

Ahmed J, Shivhare US & Raghavan GSV. 2004. Thermal degradation kinetics of anthocyanin and visual colour of plum puree. European Food Research and Technology 218(6):525-528.

Bates DM, & Watts, D.G. . 1988. Nonlinear regression analysis and its applications. New York: Wiley.

Cemeroglu B, Velioglu S & Isik S. 1994. Degradation Kinetics of Anthocyanins in Sour Cherry Juice and Concentrate. Journal of Food Science 59(6):1216-1218.

Dolan KD, Yang L & Trampel CP. 2007. Nonlinear regression technique to estimate kinetic parameters and confidence intervals in unsteady-state conduction-heated foods. Journal of Food Engineering 80(2):581-593.

Efron B. 1979. Bootstrap Methods: Another Look at the Jackknife. The Annals of Statistics 7(1):1-26.

Efron B. 1987. Better Bootstrap Confidence Intervals. Journal of the American Statistical Association 82(397):171-185.

Felsenstein J. 1985. Confidence Limits on Phylogenies: An Approach Using the Bootstrap. Evolution 39(4):783-791.

Lenz MK & Lund DB. 1977. The lethality-fourier number method: confidence intervals for calculated lethality and mass-average retention of conduction-heating, canned foods. Journal of Food Science 42(4):1002-1007.

Mishra DK, Dolan KD & Yang L. 2008. Confidence Intervals for Modeling Anthocyanin Retention in Grape Pomace during Nonisothermal Heating. Journal of Food Science 73(1):E9-E15.

Montgomery DP, EA; Vining, GG. . 2006. Introduction to linear regression analysis.,4th edition. ed.: John Wiley & sons, inc.

Nasri H, Simpson R, Bouzas J & Torres JA. 1993. Unsteady-state method to determine kinetic parameters for heat inactivation of quality factors: Conduction-heated foods. Journal of Food Engineering 19(3):291-301.

Wendie L. Claeys LRL, Marc E. Hendrickx 2001. Formation kinetics of hydroxymethylfurfural, lactulose and furosine in milk heated under isothermal and non-isothermal conditions. Journal of Dairy Research 68:287-301.

5 Conclusion

Many low- and intermediate moisture foods are processed nonisothermally at high temperatures that require attention. Based on the estimated temperature-dependent thermophysical properties, the thermal conductivity and specific heat of grape pomace increased about 45% and 37% respectively, from 25°C to 121°C. When heating for 10 min at 126.7 °C, anthocyanins were degraded up to 32%. The kinetic parameters k_r = 0.058 min^{-1} and E = 75.34 kJ/g mol were very close to those estimated by Lai (2003), who used a 1:3 grape pomace: wheat flour mixture and heated the mixture in steel cells dipped in heated oil. The fact that the estimates in the present study are so close to Lai's, who used a different procedure, strengthens the reliability of the estimates. Although the moisture parameter estimate b = 1.9 MC_{wb}^{-1} was smaller than Lai's b = 5.28 (MC_{wb}^{-1}), the trend of the degradation rate k_r increasing with increasing moisture was the same in both studies. Joint confidence region for the parameters showed a good way to represent the correlation between the two parameters.

Asymptotic confidence band and prediction band on predicted Y (anthocyanin retention) gave an approximate estimate of the error on estimated parameters, whereas bootstrap method provided narrow confidence interval for parameters, and bootstrap confidence band and prediction band for anthocyanin retention. Industrial processes for food manufacturing involve many variables that can be estimated using multiple parameter estimation method. Process design to improve food quality and ensure safety would be enhanced by knowledge of the

actual dynamic kinetic parameters of the desired components, such as nutraceuticals (quality) or pathogens (safety). Better estimates of the true error would also reduce experimental effort and cost. The method showed in this study will be a beneficial tool for future researchers involved in kinetic parameter estimation of nutraceuticals in low- and intermediate-moisture food heated at high temperatures.

Appendix A

ORAC Results

Sample number	Heating time (min)	ORAC μmol TE/g TS
1	0	16.18
2	0	18.50
3	0	17.63
4	58	15.98
5	58	16.02
6	58	16.46
7	58	17.77
8	58	18.90
9	58	17.94

One-way ANOVA

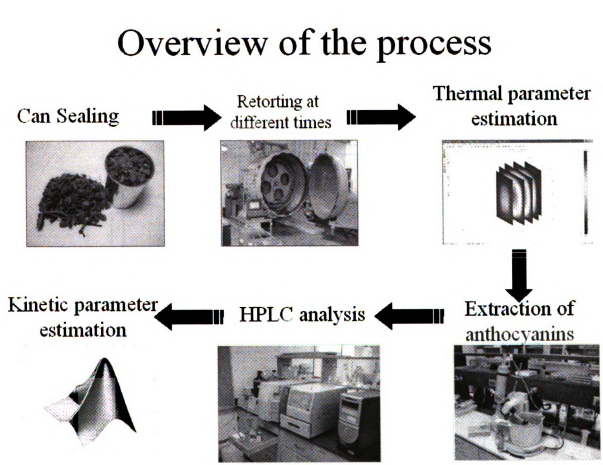
Dependent Variable: ORAC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	6.06586667	3.03293333	5.07	0.0514
Error	6	3.59193333	0.59865556		
Corrected Total	8	9.65780000			

The p value ($p>0.05$, at 95% of confidence level) suggests that there is no significant difference in mean value of ORAC in raw grape pomace and in retorted grape pomace. Hence, it is concluded that there is no degradation in the ORAC of grape pomace as a result of heating the canned grape pomace in retort.

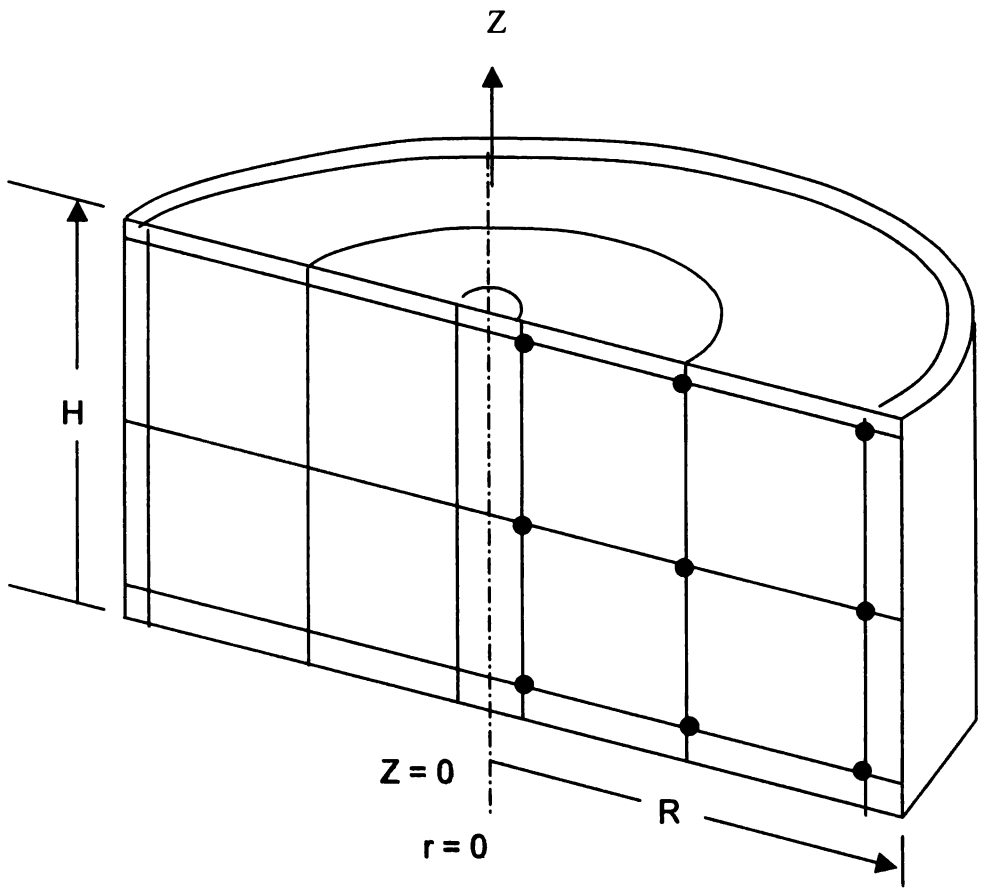
Appendix B

Flow chart of overall process



Appendix C

3-point Gauss integration. Figure represents half of the can size.



Gauss points normalized from 0 to 1 are;

1. 0.112701665379
2. 0.5
3. 0.887298334261

Appendix D

Time (sec)	Can Temperature (°C)			Retort Temperature (°C)
	1	2	3	
0	24.68	25.53	22.34	39.13
10	24.52	25.56	25.36	39.02
20	24.71	25.61	25.51	39.16
30	24.65	25.79	25.61	39.13
40	24.66	25.61	25.31	38.94
50	24.63	25.65	25.41	39.24
60	24.82	25.74	25.56	40.03
70	24.79	25.69	25.67	42.63
80	24.83	25.83	25.71	45.16
90	24.96	25.86	25.78	48.06
100	24.94	25.95	25.78	50.59
110	24.99	26.16	26.17	53.61
120	25.56	27.19	26.56	74.06
130	25.82	27.29	26.48	80.19
140	25.94	27.22	27.02	84.68
150	25.87	27.25	26.84	87.01
160	25.96	27.15	26.62	89.31
170	26.01	27.06	26.87	90.81
180	26.18	27.24	27.13	92.49
190	26.17	27.17	27.07	93.96
200	26.30	27.23	27.22	95.21
210	26.54	27.29	27.35	96.59
220	26.58	27.49	27.56	98.01
230	26.85	27.80	27.82	99.41
240	26.93	27.73	27.97	100.59
250	27.00	28.02	28.08	101.52
260	27.11	28.13	28.32	102.35
270	27.46	28.19	28.54	103.12
280	27.71	28.41	28.86	103.76
290	27.87	28.53	29.02	104.17
300	27.94	28.88	29.38	104.56
310	28.39	29.02	29.61	105.02
320	28.69	29.27	29.96	105.34
330	29.04	29.64	30.29	107.57
340	29.43	30.02	30.79	109.71
350	29.96	30.42	31.30	111.83
360	30.15	30.93	31.73	113.53
370	30.72	31.39	32.31	115.36
380	31.21	31.71	32.81	116.87
390	31.86	32.33	33.46	118.58
400	32.44	32.84	33.99	119.98
410	32.92	33.41	34.75	121.54
420	33.72	34.17	35.52	123.06

Time (sec)	Can Temperature (°C)			Retort Temperature (°C)
	1	2	3	
430	34.36	34.91	36.31	124.51
440	35.03	35.46	36.87	125.84
450	35.76	36.19	37.81	127.18
460	36.61	37.08	38.82	128.48
470	37.41	37.60	39.56	127.02
480	38.37	38.41	40.58	125.65
490	39.30	39.44	41.70	125.18
500	40.39	40.45	42.69	127.11
510	41.27	41.36	43.68	128.69
520	42.36	42.28	44.83	127.98
530	43.57	43.55	46.30	126.73
540	44.46	44.63	47.08	126.04
550	45.78	45.63	48.49	126.63
560	47.03	47.01	49.82	128.65
570	48.31	48.15	51.08	128.57
580	49.52	49.31	52.31	127.53
590	50.79	50.69	53.64	126.67
600	52.44	52.29	55.21	126.63
610	53.67	53.65	56.44	127.74
620	54.92	55.02	57.68	128.32
630	56.44	56.42	59.09	127.71
640	58.17	58.09	60.72	126.92
650	59.51	59.62	62.11	126.76
660	60.84	60.96	63.33	127.34
670	62.45	62.63	64.72	128.14
680	63.86	64.04	66.03	127.79
690	65.27	65.41	67.31	127.22
700	66.72	66.85	68.58	126.86
710	68.37	68.54	70.08	127.23
720	69.53	69.79	71.14	127.74
730	71.12	71.26	72.51	127.87
740	72.48	72.69	73.76	127.38
750	73.91	74.08	75.06	127.09
760	75.27	75.41	76.21	127.07
770	76.51	76.80	77.42	127.49
780	78.00	78.13	78.69	127.70
790	79.21	79.41	79.81	127.51
800	80.54	80.58	80.91	127.26
810	81.71	81.76	81.92	127.24
820	83.08	83.03	83.17	127.30
830	84.16	84.14	84.17	127.51
840	85.20	85.14	85.07	127.57
850	86.43	86.31	86.16	127.39
860	87.50	87.26	87.13	127.29
870	88.44	88.21	87.95	127.28

Time (sec)	Can Temperature (°C)			Retort Temperature (°C)
	1	2	3	
880	89.44	89.20	88.89	127.36
890	90.56	90.26	89.89	127.52
900	91.43	90.98	90.71	127.48
910	92.37	91.93	91.56	127.27
920	93.22	92.85	92.37	127.31
930	94.06	93.63	93.18	127.35
940	94.83	94.38	93.91	127.46
950	95.61	95.12	94.64	127.43
960	96.52	95.92	95.48	127.33
970	97.15	96.59	96.08	127.32
980	97.82	97.18	96.74	127.32
990	98.71	97.93	97.56	127.37
1000	99.17	98.56	98.08	127.30
1010	99.81	99.12	98.71	127.31
1020	100.48	99.73	99.35	127.28
1030	101.02	100.58	100.12	127.26
1040	101.61	100.88	100.53	127.29
1050	102.25	101.52	101.18	127.28
1060	102.79	101.98	101.78	127.24
1070	103.31	102.32	102.17	127.28
1080	103.76	102.90	102.81	127.22
1090	104.24	103.40	103.29	127.22
1100	104.72	103.88	103.84	127.26
1110	105.14	104.26	104.26	127.26
1120	105.77	104.65	104.92	127.36
1130	105.98	105.09	105.20	127.29
1140	106.49	105.56	105.67	127.26
1150	106.83	105.85	106.11	127.21
1160	107.11	106.22	106.55	127.18
1170	107.55	106.76	107.07	127.29
1180	107.83	106.96	107.31	127.25
1190	108.20	107.25	107.68	127.26
1200	108.67	107.66	108.19	127.26
1210	108.87	107.97	108.46	127.14
1220	109.15	108.27	108.80	127.16
1230	109.48	108.59	109.18	127.25
1240	109.73	109.01	109.47	127.21
1250	110.08	109.19	109.86	127.27
1260	110.41	109.47	110.14	127.16
1270	110.58	109.76	110.48	127.15
1280	110.84	110.08	110.78	127.19
1290	111.19	110.34	111.19	127.25
1300	111.41	110.52	111.42	127.19
1310	111.65	110.82	111.73	127.13
1320	111.87	111.17	111.98	127.11

Time (sec)	Can Temperature (°C)			Retort Temperature (°C)
	1	2	3	
1330	112.12	111.31	112.33	127.17
1340	112.33	111.72	112.54	127.15
1350	112.58	111.89	112.79	127.17
1360	112.65	112.09	113.07	127.16
1370	112.89	112.34	113.26	127.13
1380	113.15	112.61	113.56	127.15
1390	113.37	112.76	113.78	127.14
1400	113.76	112.79	113.86	127.10
1410	113.72	113.16	114.28	127.16
1420	113.86	113.31	114.46	127.12
1430	114.26	113.41	114.74	127.11
1440	114.17	113.69	114.89	127.10
1450	114.41	113.84	115.11	127.13
1460	114.53	114.14	115.30	127.13
1470	114.80	114.19	115.54	127.21
1480	114.92	114.35	115.63	127.12
1490	114.97	114.54	115.87	127.11
1500	115.20	114.59	116.02	127.06
1510	115.32	114.92	116.21	127.13
1520	115.43	115.09	116.39	127.15
1530	115.54	115.23	116.57	127.01
1540	115.74	115.39	116.67	127.09
1550	115.78	115.52	116.82	127.10
1560	115.97	115.63	116.94	127.07
1570	116.14	115.76	117.04	127.09
1580	116.26	115.88	117.22	127.13
1590	116.37	115.99	117.37	127.13
1600	116.44	116.17	117.47	127.08
1610	116.62	116.30	117.71	127.12
1620	116.66	116.36	117.75	127.07
1630	116.79	116.56	117.95	127.03
1640	116.84	116.63	117.92	127.21
1650	117.03	116.76	118.18	127.07
1660	117.06	116.86	118.23	127.05
1670	117.21	116.98	118.39	127.08
1680	117.21	116.82	118.47	126.89
1690	117.29	116.89	118.53	127.61
1700	117.34	116.99	118.63	127.46
1710	117.48	117.09	118.76	126.96
1720	117.51	117.21	118.82	127.18
1730	117.63	117.34	118.95	126.48
1740	117.70	117.42	119.03	126.31