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PREDICTION AND CONTROL OF VITAMIN C LOSS IN SPACEFLIGHT FOODS

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PREDICTION AND CONTROL OF VITAMIN C LOSS IN SPACEFLIGHT FOODS

A Dissertation Presented

by

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DEDICATION

I would like to dedicate this thesis to everyone who had to put up with my shenanigans throughout my life. If you feel like you were a victim of this, please consider this thesis as a token of appreciation to you. My sincere apologies for the inconvenience I partook on your life, especially my parents.

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I would like to give a huge thanks to my parents, siblings, and close family members. I would also like to thank my thesis committee and NASA grant team members, graduate funding sources, organizations/affiliations, and anyone/anything that impacted my academic success financially or personally.

ABSTRACT

PREDICTION AND CONTROL OF VITAMIN C LOSS IN SPACEFLIGHT FOODS

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Shelf stable foods that require no refrigeration or freezing are the predominant food source for astronauts. Due to its high impact on astronauts' health, it is crucial to know if the astronauts are getting all the necessary nutrients from shelf stable foods, specifically vitamins. With limited knowledge on vitamin degradation in spaceflight foods during storage and processing, our team decided to tackle this issue focusing on unstable vitamins, which included vitamin C (ascorbic acid) and vitamin B1 (thiamine); however, this thesis will specifically focus on vitamin C.

A two-year storage and retort processing study was conducted on 5 different foods (i.e. sugar snap peas, strawberries, and rhubarb applesauce at three different pH levels) to determine the vitamin degradation kinetics and this information was used to determine a mathematical model reliability to predict and control vitamin C degradation during long term storage and retort processing using experimental data. Validation and improvement of model was implemented based on experimental data.

For the storage study, each food was retorted and freeze dried to make it shelf stable according to NASA specifications. The foods were stored at five constant temperatures (-20, -80, 4, 20, and 37 °C). Over a two-year period, samples were periodically pulled and HPLC analysis was used to measure vitamin C. With vitamin C

measured at two experimental points, degradation parameters, k_{Tref} and c, were determined to make predictive degradation curves for each food, process, and positive temperature for vitamin C using the endpoints method model. When the two-year storage study was completed, the predictive degradation curves were compared to experimental data. Additionally, the combined first order kinetics model incorporating all experimental data was used to determine degradation parameters: $C_{asymptote}$, k_{Tref}, and c. With both models and physiochemical properties (i.e. pH and moisture content) of each food, two databases were created to determine degradation parameters for vitamin C with inputting a pH, moisture content, and storage temperature to retrieve estimated degradation parameters.

The retort processing study focused on degradation pre-storage and the endpoints method model was adjusted to predict nonisothermal data compared to the isothermal data utilized during the two-year storage study. The results showed that the endpoints method model was effective for nonisothermal and isothermal predictions. The physiochemical property databases created from the two-year storage study provided a helpful complimentary tool to estimate degradation parameters without doing a storage study. However, further improvements to functions used to determine degradation parameters is crucial to make database more accurate. The knowledge obtained during these studies will help ensure that NASA's astronauts are getting all the necessary nutrients needed at any time to maintain health and wellness in space.

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LIST OF COMMON ABBREVIATIONS AND VARIABLES

AA: ascorbic acid

DHA: dehydroascorbic acid

Asymp: asymptotic

$C(t)$: momentary concentration at time t

EDTA: ethylenediaminetetraacetic acid

n: reaction's order

$k[T(t)]$: the momentary rate constant

ODE: ordinary differential equation

T: temperature

T_{ref}: reference temperature

t: time

$T(t)$: the momentary temperature

Vit C: vitamin C

WLF: Williams-Landel-Ferry

Eq/Eqs: Equation(s)

CV: coefficient of variation

RAvg: Residual average

RSD: Residual standard deviation

TP/FD: thermoprocessed/freeze dried

SP: sugar snap peas

RA: rhubarb applesauce

ST: strawberries

CHAPTER 1

INTRODUCTION

Vitamin C (Vit C), also known as L-ascorbic acid, ascorbic acid (AA), or ascorbate, is a well-studied water-soluble vitamin. When oxidized, the molecule is converted to dehydroascorbic acid (DHAA) and a myriad of other degradation products; however, DHAA is the only biologically active byproduct that can be converted back to AA. AA also has antioxidant properties along with being an essential vitamin for human consumption due to human's inability to produce the compound. In the body, it acts as a cofactor for many enzymatic and nonenzymatic reactions. Without it, collagen synthesis is impaired leading to poor oral and wound healing health. Under severe conditions, one could develop scurvy. However, this has been eradicated in the United States with vitamin supplementation and fortification in many food products, considering only 10 mg/day is needed to prevent scurvy. Many processed foods and beverages contains 100% DV of vitamin C, which is equivalent to 60 mg AA/day. AA is also naturally high in many fruits and vegetables. AA also has been associated with preventing and treating many diseases, such as cardiovascular diseases, cancer, diabetes mellitus, cardiovascular diseases, the common cold, and many more (Chambial, Dwivedi, Shukla, John, & Sharma, 2013; Naidu, 2003). However, many of these claims have been challenged and should not be a dependable source to combat these issues alone.

Moreover, vit C is one of the least stable vitamins needed for human consumption. Where many intrinsic and extrinsic properties affect vit C stability, such as pH, light, oxygen levels, temperature, food matrix, packaging, and processing methods. Oxygen is a key player in vit C instability. The location can determine how significant

this can be such as being dissolved or headspace oxygen with the dissolved oxygen typically causing more rapid degradation (Zerdin, Rooney, & Vermuë, 2003). The pH is another variable associated with vit C stability. Where it has been reported that higher acidic foods can improve vit C stability. Temperature is also a large influencer on vit C stability. From many sources, it is reported lower temperatures provide better stability than higher temperatures (Awuah, Ramaswamy, & Economides, 2007). Matrix type is inclusive of all the general intrinsic properties affecting vitamin C stability. One of the main concerns are metal catalysts and water activity. Iron is typically the common culprit, but copper, zinc, cobalt, any other essential metals or unwanted metals in the diet can also increase vit C loss. Water activity or moisture content follow a similar trend to where higher amounts can enhance the degradations of vit C. There are many processing methods affecting vit C loss, but this study will only focus on freeze drying and retort processing. Freeze drying is a low heat treated drying method that has been supported in many studies to preserve nutrients better than other drying methods. The lower water activity from freeze drying helps mitigate microbial growth, which is vital for prolonging food storage (Santos & Silva, 2008; Vergeldt et al., 2014). Retort thermoprocessing provides a harsher form of processing due to higher temperature exposure. These two processing methods are two conditions that can highly impact vit C stability, especially under retort processing.

Although retort processing can be destructive to vitamins, processing foods are essential for food preservation and safety; however, it is still important for crew members to have an adequate supply of vit C in their foods throughout long-duration missions.

Therefore, a balance of food safety and nutrient stability must be achieved without compromising food safety.

In summary, a myriad of NASA spaceflight food recipes was produced and stored under five temperatures for two years, and the degradation kinetics of vitamin C were systematically determined with various models. This information was used to predict and control vitamin C loss in spaceflight foods, and its reliability was assessed and validated. The results were analyzed based on the nature of different spaceflight foods to develop guiding principles on how to minimize vitamin degradation in spaceflight foods. The project provided critical information that can be used to produce more nutritious shelf-stable spaceflight foods to help ensure health and wellness of astronauts in space.

CHAPTER 2

LITERATURE REVIEW: MODELING THE DEGRADATION KINETICS OF ASCORBIC ACID

2.1. Abstract

Most published reports on ascorbic acid (AA) degradation during food storage and heat preservation suggest that it follows first-order kinetics. Deviations from this pattern include Weibullian decay, and exponential drop approaching finite non-zero retention. Almost invariably, the degradation rate constant's temperature-dependence followed the Arrhenius equation, and hence the simpler exponential model too. A formula and freely downloadable interactive Wolfram Demonstration to convert the Arrhenius model's energy of activation, E_a, to the exponential model's c parameter, or vice versa, are provided. The AA's isothermal and non-isothermal degradation can be simulated with freely downloadable interactive Wolfram Demonstrations in which the model's parameters can be entered and modified by moving sliders on the screen. Where the degradation is known *a priori* to follow first or other fixed order kinetics, one can use the endpoints method, and in principle the successive points method too, to estimate the reaction's kinetic parameters from considerably fewer AA concentration determinations than in the traditional manner. Freeware to do the calculations by either method has been recently made available on the internet. Once obtained in this way, the kinetic parameters can be used to reconstruct the entire degradation curves and predict those at different temperature profiles, isothermal or dynamic. Comparison of the predicted concentration ratios with experimental ones offers a way to validate or refute the kinetic model and the assumptions on which it is based.

2.2. Introduction

The chemical mechanisms and kinetics of vitamins degradation during food processing and storage has been thoroughly investigated for decades and there is a large body of literature on the subject. Recently, interest in vitamins loss kinetics has been revived due to NASA's preparations for long interplanetary human flights, and vitamin C has been prominent among them. The degradation of ascorbic acid follows two major pathways (Manso, Oliveira, Oliveira, & Frías, 2001; Verbeyst, Bogaerts, Van der Plancken, Hendrickx, & Van Loey, 2013; Vieira, Teixeira, & Silva, 2000; Yuan & Chen, 1998). In one, known as the 'aerobic pathway' the L-ascorbic acid (AA) is oxidized to dehydroascorbic acid (DHAA or DHA), which then can disintegrate in different ways. In the other, known as the 'anaerobic pathway', the ascorbic acid disintegrates without being oxidized first so the intermediate degradation products do not include DHAA. However, the two degradation mechanisms can operate simultaneously albeit at different rates. Thus, whenever the aerobic pathway plays a role, the DHAA concentration first rise and then drops as it too disintegrates and forms other compounds. Also, since DHAA is functionally a vitamin, modeling the nutritional loss of vitamin C in foods is not as straightforward as that of other vitamins where only their intact molecule has the desired biological activity. In this article, we will only address the degradation kinetics of the original AA molecules, which is frequently the only form of the vitamin that is monitored in industrial food processing and storage studies.

Since ascorbic acid is an antioxidant, the roles of oxygen, oxidative agents and catalysts presence in its degradation mechanisms and kinetics have also received considerable attention (Fustier, St-Germain, Lamarche, & Mondor, 2011; Odriozola-

Serrano, Soliva-Fortuny, & Martín-Belloso, 2009; Van Bree et al., 2012; Zerdin et al., 2003), including in non-food model systems (Curtin et al, 2014). These recent publications contain extensive reference lists of pertinent earlier studies of the subject [see also (Lešková et al., 2006)].

This review does not address the nutritional aspects of vitamin C and its loss in processed and stored foods, the chemistry of its degradation, or the analytical methods of its determination in foods. Its central topic is to publish kinetic models of ascorbic acid degradation in foods and their mathematical properties. The focus is on interactive software recently posted on the internet with which these models can be used in simulations and visualization, and on the possibility of exploiting the models' mathematical properties to reduce the number of chemical determinations in storage studies.

2.3. Theoretical kinetic models of ascorbic acid degradation

2.3.1. Fixed Order Kinetics

Fixed order degradation kinetics is described by the rate equation (Boekel, 2008):

$$\frac{\mathrm{d}C(t)}{\mathrm{d}t} = -k[T(t)]C(t)^n \qquad (1)$$

where in our case $C(t)$ is the momentary AA concentration at time t, $T(t)$ is the momentary temperature, $k[T(t)]$ is the momentary rate constant, and n is the reaction's order.

For first order kinetics ($n = 1$) and for constant temperature $T(t) = T$, and for the boundary condition $C(0) = C_0$, the AA's initial concentration, Eq. 1 has an analytical solution having the form

$$C(t)/C_0 = \mathrm{Exp}[-k(T)t] \qquad (2)$$

For n^{th} order kinetics ($n \neq 1$) the isothermal solution of Eq. 1 is

$$C(t)/C_0 = 1 - k(T)\,((n-1)t)^{1/(1-n)} \qquad (3)$$

Although rarely if ever encountered in practice, Eq. 3 implies that where $n = 0$, the concentration ratio becomes negative at $t > 1/k(T)$, and where $0 < n < 1$, it becomes a complex number at $t > 1/C_0/k(T)^{1-n}$. To avoid the occurrence of such situations, any general program for simulating and predicting a vitamin's degradation pattern, based on Eq. 1 as a model, has to automatically replace any negative or complex value of the concentration ratio by zero, which can be done with 'If' statements (Peleg, Normand, & Kim, 2014). To visualize the effect, the interested reader can generate isothermal degradation patterns using the modified model with the freely downloadable interactive Wolfram Demonstration http://demonstrations.wolfram.com/KineticOrderOfDegradationReactions/. [The free Wolfram CDF Player, which runs the Demonstration (and over 10,000 other Demonstrations to date), can be downloaded following the instructions on the screen.]

2.3.2. Zero Order Kinetics

When the decay rate is very low, and the experimental concentration measurements have a scatter, the degradation curve may appear linear at least visually. Also, if such data are submitted to linear regression, the regression coefficient r^2 is likely to be high. Thus for all practical purposes the degradation can be treated as following zero order kinetics on the pertinent time scale, but probably not for long range extrapolation. Examples of what appears as zero order kinetic degradation of AA can be found in (Tiwari, O' Donnell, Muthukumarappan, & Cullen, 2009) who studied the relatively marginal effect of sonification and others (Robertson & Samaniego, 1986; Van Bree et al., 2012). However, zero order degradation kinetics has also been reported for a

substantial loss of AA exposed to high temperatures and various levels of water activity (Laing et al, 1978).

2.3.3. First Order Kinetics

Most of the publications on AA degradation report that it followed first order kinetics, regardless of the food or medium, the temperature range and time scale (Bosch et al., 2013; Burdurlu, Koca, & Karadeniz, 2006; Cruz, Vieira, & Silva, 2008; Giannakourou & Taoukis, 2003; Johnson, Braddock, & Chen, 1995; Laing, Schlueter, & Labuza, 1978; Lee & Coates, 1999; Li, Yang, Yu, & Wang, 2016; Polydera, Stoforos, & Taoukis, 2003, 2005; Uddin, Hawlader, Ding, & Mujumdar, 2002; Van Bree et al., 2012).

2.3.4. Combined First Order Kinetics Models

Ascorbic acid has two degradation mechanisms that can occur simultaneously: the already mentioned aerobic and anaerobic pathways. Consequently, the AA's diminishing concentration in a particular food or medium is governed by two temperature-dependent rate constants, $k_{aerobic}(T)$ and $k_{anaerobic}(T)$. Thus, if both degradation pathways follow first order kinetics, then AA's isothermal disappearance is described by the model (Verbeyst et al., 2013):

$$C(t) = C_{aerobic}\, Exp(-\, k_{aerobic}\, t) + (1 - C_{aerobic})\, Exp(-\, k_{anaerobic}\, t) \qquad (4)$$

where $C(t)$ is the momentary fraction of the original AA and $C_{aerobic}$ is the fraction of the original AA concentration, which is degraded by the aerobic mechanism (where AA is oxidized to DHAA first). Also, according to these authors, when the non-oxidative mechanism's contribution is very small, i.e., $k_{anaerobic} << k_{aerobic}$ or ~ 0, the fraction $1 - C_{aerobic}$ is practically a constant. If so, Eq. 4 becomes (Vieira et al., 2000):

$$C(t) = C_{asymp} + (1 - C_{asymp})\, Exp(-\, k_{aerobic}\, t) \qquad (5)$$

where C_{asymp} is the asymptotic concentration fraction of the original AA. In other words, the degradation curve initially follows the exponential decay pattern expected from first order kinetics but then instead of the AA decaying asymptotically to zero it decays to a residual nonzero value, at least on a pertinent time scale. To simulate this isothermal degradation pattern, open the Wolfram Demonstration Simulating Ascorbic Acid Degradation at http://demonstrations.wolfram.com/SimulatingAscorbicAcidDegradation/. Examples of this Demonstration's screen displays are given in Figure 2.1.

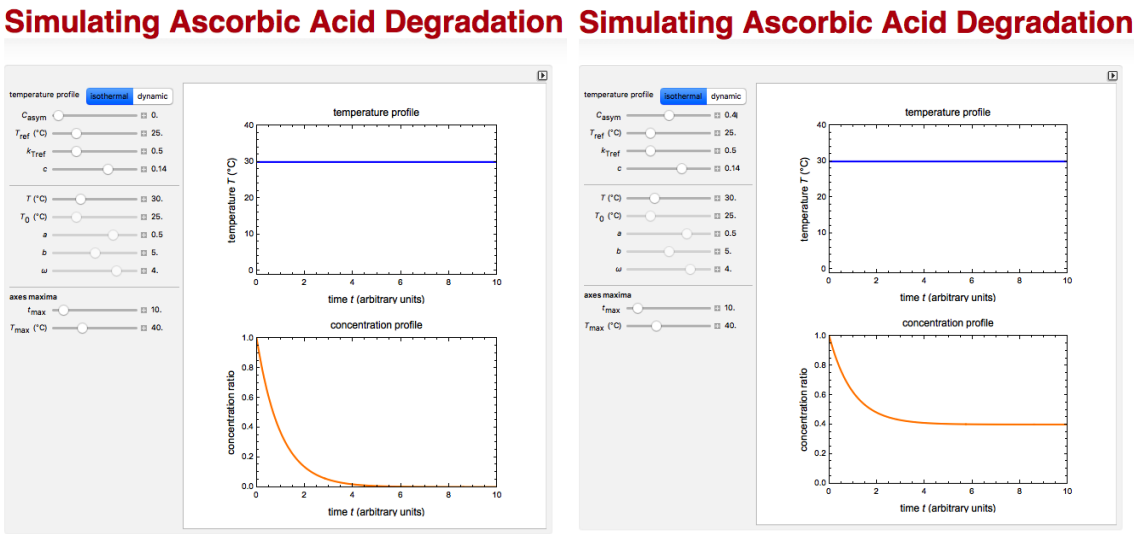


Figure 2.1. Screen displays of the Wolfram Demonstration that simulates isothermal ascorbic acid degradation. Left – Conventional first order kinetics, right – Exponential decay approaching asymptotic residual retention.

The left side of the figure shows a degradation curve that follows first order kinetics ($C_{asymp} = 0$), and the right side a curve that follows Eq. 5 as a model ($C_{asymp} > 0$). Notice that Eqs. 4 and 5 are akin to the biphasic exponential model (Boekel, 2008), which for our purpose can be written in the form (Maria G. Corradini & Peleg, 2006):

$$\text{Log } C(t) = -k_{aerobic}\, t \text{ if } t \leq t_c \text{ and } -k_{anaerobic}\, t \text{ if } t > t_c \qquad (6)$$

where t_c marks the time when the change in slope occurs and $k_{aerobic} > k_{anaerobic}$. If $k_{anaerobic} \sim 0$, it will approximate the curve produced by Eq. 5. To simulate and visualize degradation

curves with Eq. 6 as a model open the freely downloadable Wolfram Demonstration at http://demonstrations.wolfram.com/BiphasicExponentialDecayAndGrowth/. Although some published data suggest the existence a biphasic degradation pattern (Polydera et al., 2003; Verbeyst et al., 2013), whether the biphasic model (Eq. 6) has ever been tried in the study of AA degradation is unknown to the authors.

2.3.5. Weibullian Kinetics

Chemical and thermal degradation can be viewed as a failure phenomenon; for instance, a manifestation of the molecules' inability to remain intact in the particular environment. Thus the degradation curve, depicting the diminishing concentration vs. time relationship, is basically a survival curve, the cumulative form of the disintegration events' temporal distribution. When the degradation curve is expressed in terms of a concentration ratio, its local slope, having time reciprocal units, is the process's *rate*. In many diverse and unrelated physical breakage and disintegration phenomena the disintegration events have a Weibull temporal distribution, which has been known as the Rosin-Rammler distribution in particulates size reduction. When adapted for isothermal chemical degradation it can be written in the form known as the stretched exponential:

$$C(t) = \mathrm{Exp}\left[-\left(\frac{t}{t_c(T)}\right)^{m(T)}\right] \text{ or } \mathrm{Exp}[-b(T)t^{m(T)}] \qquad (7)$$

where $C(t)$ is the momentary concentration ratio, t_c(T) is a temperature-dependent characteristic time ("scale factor") or $b(T)$ a temperature-dependent rate parameter, and $m(T)$ a power (known as the "shape factor"). The power $m(T)$ in Eq. 7 is usually a weak function of temperature and can be treated as a constant, i.e., $m(T) \sim m$ in many applications. Notice that where $m(T) = 1$, Eq. 7 describes first order degradation kinetics. Also, depending on the degradation data scatter, the fixed order kinetics (Eq. 2) and the

Weibullian model (Eq. 7) can be used interchangeably when *m* or *n* is between about 0.8 and 1.2. This can be seen in the interactive Wolfram Demonstration http://demonstrations.wolfram.com/FitOfFirstOrderKineticModelInDegradationProcesses/

Application of the Weibullian model to AA degradation has been reported by (Maria G. Corradini & Peleg, 2004, 2006; Derossi, De Pilli, & Fiore, 2010; Manso et al., 2001; Odriozola-Serrano et al., 2009; Tiwari et al., 2009; Zheng & Lu, 2011).

2.4. The role of temperature

2.4.1. The Arrhenius equation and exponential model

The temperature-dependence of the rate constant, however defined, has been traditionally described by the Arrhenius equation, which can be written in the form:

$$k(T) = k(T_{\text{ref}})\text{Exp}\left[\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right] \qquad (8)$$

where $k(T)$ is the rate constant in the pertinent concentration per time units at temperature T in °K and $k(T_{\text{ref}})$ is the rate constant at a reference temperature T_{ref} in °K. E_a according to this model is the "energy of activation", usually expressed as kJ or kcal per mole, and R is the Universal Gas Constant in commensurate units.

It has been demonstrated (Peleg & Normand, 2015; Peleg, Normand, & Corradini, 2012; Peleg et al., 2014) that without sacrificing the fit, the Arrhenius equation can be replaced by the simpler exponential model:

$$k(T) = k(T_{\text{ref}})\ \text{Exp}[c(T\text{-}T_{\text{ref}})] \qquad (9)$$

where T and T_{ref} are in °C and c is a constant having $°C^{-1}$ units. Demonstration of the interchangeability of the two models can be viewed in the Wolfram Demonstration http://demonstrations.wolfram.com/ArrheniusVersusExponentialModelForChemicalReac

tions/. The advantage of the exponential model over the Arrhenius equation, apart from its obvious simplicity, is that it does not require one to assume that the activation energy of chemical reactions and biological processes in foods is universally temperature-independent, an assumption yet to be confirmed experimentally. The interchangeability of the two models should not come as a surprise. This is revealed by the Taylor series expansion of $k(T)$ when expressed by the two models at T_{ref}. It shows that the first two terms are identical and that at temperatures pertinent to food storage and processing, the series converges very rapidly (Peleg et al., 2012). Consequently, one can convert published E_a values into c values and vice versa using the formula:

$$c \approx \frac{E_a}{R(T_{ref}+273.16)^2} \quad \text{or} \quad E_a \approx c\mathrm{R}(T_{ref}+273.16)^2 \qquad (10)$$

To do the conversion online one can use the Wolfram Demonstration http://demonstrations.wolfram.com/ExponentialModelForArrheniusActivationEnergy/. Examples of the interchangeability of the two models when applied to ascorbic acid's published degradation data in different foods at different temperatures are given in Figure 2.2. These examples demonstrate that as long as the reference temperature is in a pertinent temperature range, its choice has no discernible effect on the two models' fit.

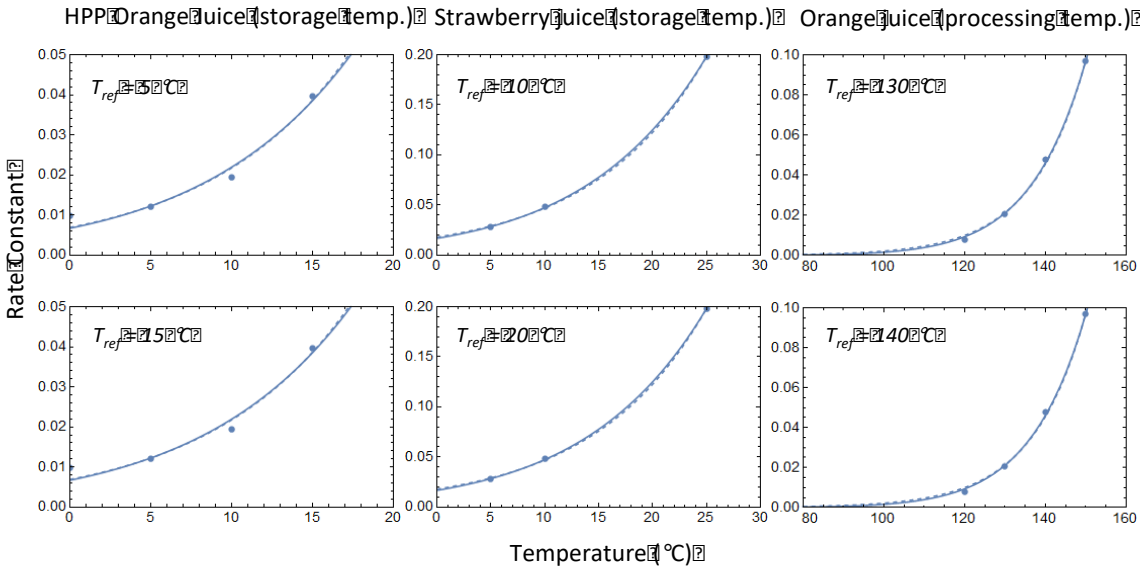


Figure 2.2. The interchangeability of the Arrhenius equation (Eq. 6 – solid curve) and exponential model (Eq. 7- dashed curve) for describing ascorbic acid degradation. The experimental data, left to right, are from (Polydera et al., 2003), (Derossi et al., 2010), and (Polydera et al., 2005), respectively.

Reported E_a values for ascorbic acid degradation in various foods are mostly in the range of 10-80 kJ/mole (or about 2 -18 kcal/mole) which for T_{ref} = 25°C correspond to c values of 0.0135-0.081°C^{-1}. The reported values in frozen vegetables (Cruz et al., 2008) were 130-150 kJ/mole (or 31-36 kcal/mole) which for T_{ref} = -5°C correspond to c values of 0.217-0.250°C^{-1}. Notice that the physical meaning of any reported E_a value obtained from an Arrhenius plot's slope is unclear, unless confirmed by independent experimental determination or compelling theoretical arguments that the Arrhenius model indeed applies. It is most likely that the same experimental $k(T)$ vs. T data fitted by the Arrhenius equation could also be successfully fitted with the Eyring-Polanyi model (Cisse, Vaillant, Acosta, Dhuique-Mayer, & Dornier, 2009), and most probably by several empirical models as well – see below. To view the almost perfect interchangeability of the Arrhenius and

Eyring-Polanyi models open http://demonstrations.wolfram.com/ArrheniusVersusEyringPolanyiModel/.

2.4.2. Alternative temperature-dependence models

The Arrhenius equation has been by far the most widely used model to describe the temperature-dependence of the degradation rate constant of ascorbic acid. Other models, adapted from microbial inactivation, have been the log-linear relationship, which has produce the *D* and *z* values (Castro, Teixeira, Salengke, Sastry, & Vicente, 2004; Johnson et al., 1995), variants of the Belerādek's also known as Ratkowsky's 'square root' model (Valdramidis, Cullen, Tiwari, & O'Donnell, 2010) and the logarithmic-exponential model (Maria G. Corradini & Peleg, 2004, 2006; Derossi et al., 2010). The WLF equation imported form the polymer science literature has also been considered (Giannakourou & Taoukis, 2003). This model implies that the rate of chemical degradation reactions in a food is primarily determined by how far the food is from its 'glass transition temperature', T_g, which is rarely uniquely defined.

To visualize the simplest version of the 'square root' model open http://demonstrations.wolfram.com/SquareRootModelForRatesOfMicrobialGrowthOrInactivation/, to visualize the logarithmic-exponential model, open http://demonstrations.wolfram.com/WeibullianInactivationRateAsAFunctionOfTemperature/, and to visualize the WLF equation), open http://demonstrations.wolfram.com/WilliamsLandelAndFerryEquationComparedWithActualAndUniversal/. A discussion of the merits and limitations of these and other temperature-dependency models can be found in (Peleg et al., 2012).

2.4.3. Non-isothermal degradation

In heat processing or dynamic storage of foods, where the temperature varies with time, i.e., $T(t) \neq$ constant, Eq. 1 has an analytic (algebraic) solution only for a few combinations of the values of n and the temperature history's profile. When the temperature-dependence of the rate constant follows the exponential model (and hence the Arrhenius equation) it assumes the form:

$$\frac{\mathrm{d}C(t)}{\mathrm{d}t} = -k_{Tref}\, Exp[c[(T(t) - T_{ref})]C(t)^n \qquad (11)$$

where $C(t)$ is the momentary concentration ratio and $C(0) = 1$ is the boundary condition. Regardless of the value of n, Eq. 11 is an ordinary differential equation (ODE) and can be rapidly solved numerically with Mathematica® and other advanced mathematical programs, even for elaborate temperature profiles that might include "if" statements (Peleg et al., 2014). Examples of dynamic degradation curves of reactions that follow fixed order kinetics can be generated with the Wolfram Demonstration *http://demonstrations.wolfram.com/NonisothermalDegradationKinetics/* whose screen display is shown in Figure 2.3.

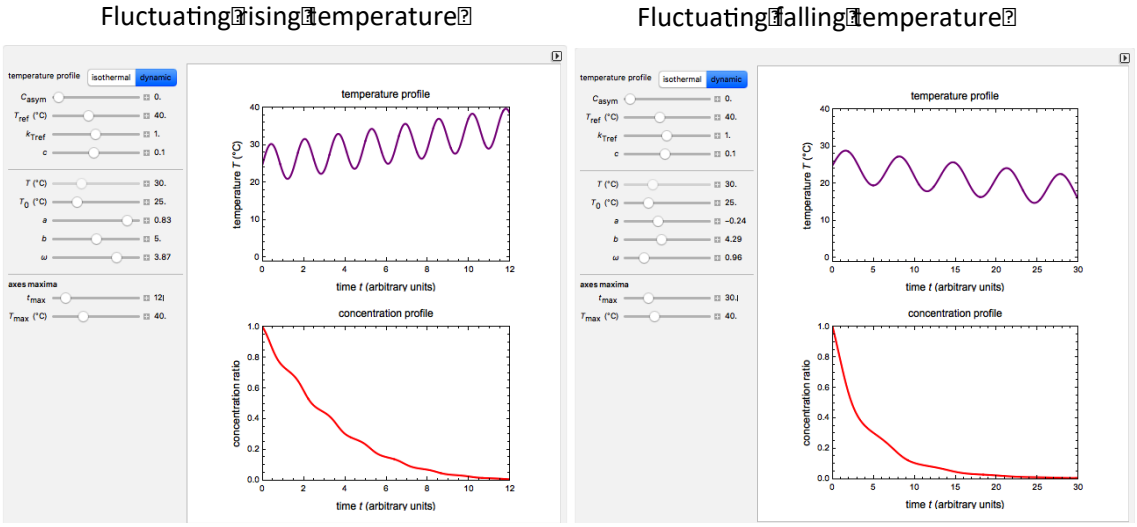


Figure 2.3. Simulated non-isothermal (dynamic) degradation curves that follow fixed order kinetics using Eq. 9 as a model.

When it comes to non-isothermal degradation curves of ascorbic acid, which under isothermal conditions are governed by Eq. 5 as a model, i.e., where there is an asymptotic retention ratio C_{asymp}, Eq. 11 no longer applies and needs to be replaced. In principle at least, the isothermal Eq. 5 can be converted into a general dynamic rate equation by assuming the following:

1. The asymptotic concentration ratio, C_{asymp}, is actually or practically temperature-independent.
2. In a pertinent temperature range, the temperature-dependence of the rate constant $k(T)$ still follows the exponential model (and therefore the Arrhenius equation), and
3. The momentary dynamic degradation rate, $dC(t)/dt$, is the isothermal rate at the momentary temperature, $T(t)$, at a time $t^*(t)$, which corresponds to the momentary concentration, $C(t)$. If all three assumptions hold, then

$$\frac{dC(t)}{dt} = -k_{T_{ref}}(1 - C_{asymp})Exp[-Exp[c\big(T(t) - T_{ref}\big)k_{T_{ref}}t^*(t) + c(T(t) - T_{ref}) \qquad (12)$$

where

$$t^*(t) = -Ln[C(t) - C_{asymp})/(1 - C_{asymp})]/(\, k_{T_{ref}}\, Exp[c\big(T(t) - T_{ref}\big)]) \qquad (13)$$

Despite its cumbersome mathematical appearance, this model too is an ordinary differential equation (ODE). Consequently, this model equation can be solved numerically to describe degradation patterns under almost any conceivable temperature history using Mathematica® and other advanced mathematical programs. Eqs. 12's validity as a model of AA's dynamic degradation kinetics is yet to be confirmed experimentally. Also, notice that Eq. 11 is a special case of Eqs. 12 and 13 where $C_{asymp} = 0$. The issue of whether and how the value of C_{asymp} can be assessed a priori is yet to be fully resolved, see below.

However, whenever C_{asymp} is known, or can be assumed on the basis of published data, the model expressed in Eqs. 12 and 13 can be used to simulate both isothermal and dynamic degradation curves of ascorbic acid including in scenarios where it has no residual retention. An interactive Wolfram Demonstration that simulates isothermal and dynamic AA degradation can be found at http://demonstrations.wolfram.com/SimulatingAscorbicAcidDegradation/. Examples of its screen displays are given in Figure 2.4.

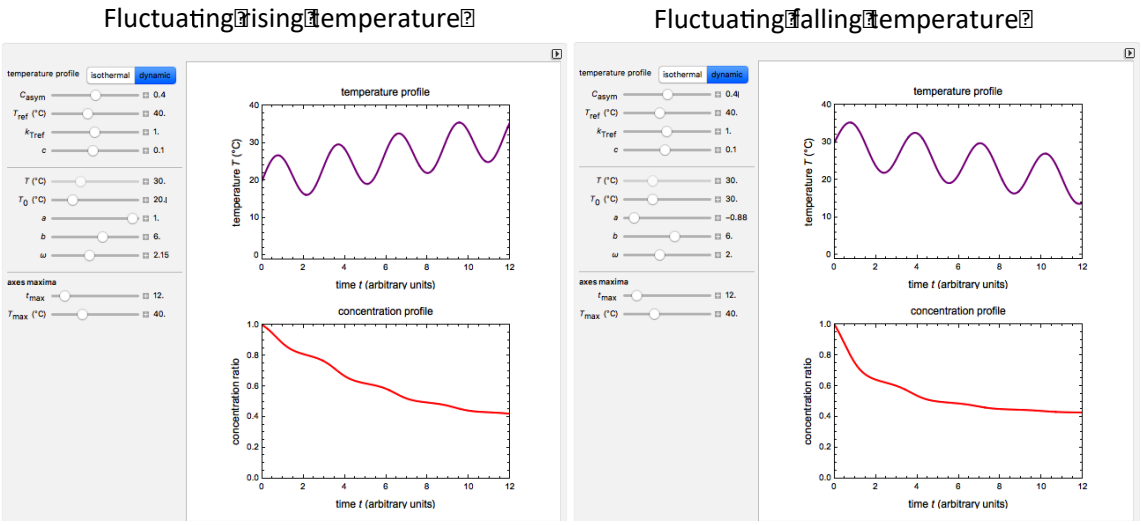


Figure 2.4. Simulated non-isothermal (dynamic) degradation curves that approach asymptotic residual retention using Eq. 13 as a model.

In principle, similar assumptions can be made for the AA's dynamic degradation patterns that follow the Weibullian model (Eq. 7). In that case, however, there is no asymptotic residual concentration ratio, but as before, the rate parameter, $b(T)$, is expected to follow the exponential model (and hence the Arrhenius equation). For convenience, the resulting rate equation can be written in the form:

$$\frac{dy(t)}{dt} = -b_{Tref}Exp[c(T(t) - T_{ref})]m\left[\frac{y(t)}{b_{Tref}Exp[c(T(t)-T_{ref})]}\right]^{\frac{m-1}{m}} \qquad (14)$$

where $y(t)$ = Ln[$C(t)$], i.e., the natural logarithm of the residual concentration ratio, $T(t)$ is the temperature profile's equation, and $y(0) = 0$ is the boundary condition. The actual dynamic degradation curve expressed in terms of the concentration ratio $C(t)$ vs. t would then be described by:

$$C(t) = Exp[y(t)] \qquad (15)$$

where $y(t)$ is the solution of Eq. 14. A Wolfram Demonstration that generates isothermal and dynamic Weibullian degradation patterns using the above model has been posted on the internet, open http://demonstrations.wolfram.com/WeibullianChemicalDegradation/. Again, to the best of our knowledge, application of this dynamic version of the Weibullian model to ascorbic acid degradation has not yet been reported. One can only find a similar version of the model where $b(T)$ is described by the logistic-exponential model (Maria G. Corradini & Peleg, 2006; Derossi et al., 2010). Since the Arrhenius equation, and hence the exponential model, and the log-exponential modes have a substantial region of practical overlap (Peleg, Engel, Gonzalez-Martinez, & Corradini, 2002), one can expect that these three models will produce similar dynamic degradation patterns in cases where the AA shows no asymptotic retention on the pertinent time scale.

2.5. The endpoints method to determine aa's kinetic parameters

2.5.1. The conventional method to determine kinetic degradation parameters

Traditionally, the kinetics of ascorbic acid degradation during thermal processing or storage, as that of other vitamins, has been determined from a series of experimental isothermal concentration or concentration ratio vs. time relationships at a pertinent temperature range (e.g. Burdurlu et al., 2006; Giannakourou & Taoukis, 2003; Manso et al., 2001; Van den Broeck, Ludikhuyze, Weemaes, Van Loey, & Hendrickx, 1998; Vieira

et al., 2000). Plots of the data on linear, semi-logarithmic, or other coordinates and/or linear or nonlinear regression have been used to establish the degradation reaction's kinetic order, *n*, and to determine the corresponding rate constant's temperature-dependence. Almost invariably, as stated in previous sections, this dependence has been described mathematically by the Arrhenius equation, which has been employed to predict the degradation patterns at different temperature histories, isothermal or dynamic. Although mostly successful, this methodology raises two issues. At high temperature-short time processing (HTST), especially well above 100°C as in UHT processing, obtaining isothermal temperature profiles, even approximately, is extremely difficult if not utterly impossible (Peleg et al., 2008). This problem hardly, if ever, exists in storage studies where the come-up and/or cooling times are almost always negligible relative to the "holding time". The main issue in storage studies is that the conventional procedure requires a relatively large number of samples to be stored and tested periodically, which creates a logistic issue (Peleg & Normand, 2015; Peleg, Normand, & Goulette, 2016; Peleg et al., 2014). For example, in a conventional setup of 4 storage temperatures with 4 samples pulled for analysis at each temperature, the number of concentration determinations is 16. If each analysis is performed in triplicates, the total number of samples to be actually analyzed is 48. The question that arises is whether the kinetic parameters can be estimated, from a smaller number of storage temperatures and a considerably smaller number of determinations and chemical analyses. For instance, if the needed kinetic information could be extracted from the same food stored at only 3 storage temperatures and the AA concentration is determined in triplicates *only once at each temperature* (after a sufficient time to detect the degradation), the number of tested samples would be reduced to 9. Thus

if a method to calculate the kinetic parameters from a smaller number of experimental data is found, its implementation would result in considerable savings, especially in studies where a large number of food products are investigated.

2.5.2. The endpoints method

The endpoints method is based on the tenet that when the general kinetics is known a priori or can be assumed, and the food's temperature history accurately recorded, one can use the final concentrations after two or three different heat treatments or storage temperature histories to extract the unknown kinetic parameters – see Figure 2.5.

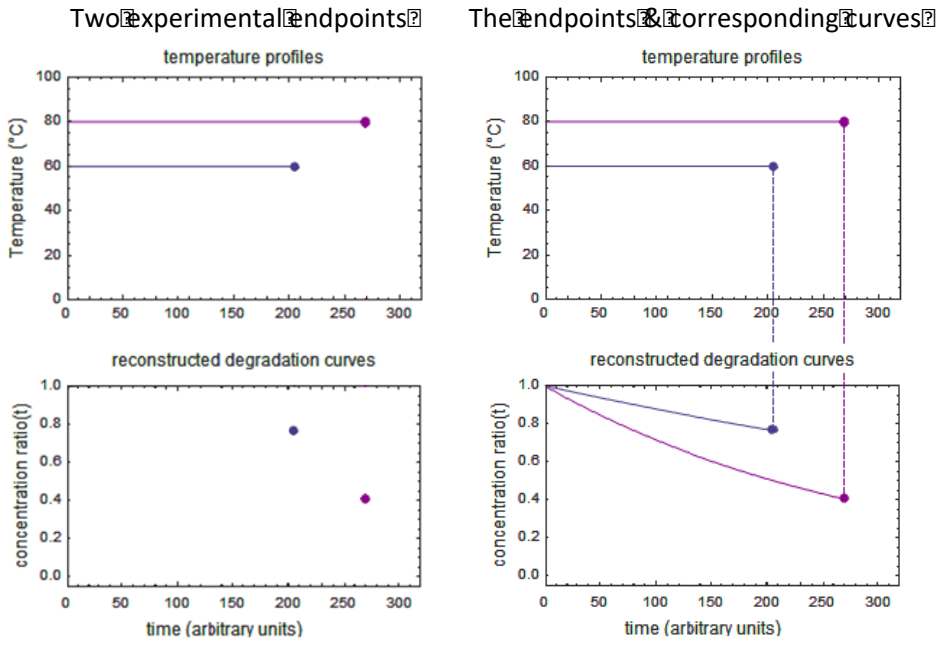


Figure 2.5. The principle of the endpoints method: Left – Two experimentally determined AA concentration ratios C_1 and C_2 at two temperatures, T_1 and T_2 at times t_1 and t_2, respectively, Right – these two endpoints ought to lie on the two corresponding degradation curves, which follow the kinetic model's equation.

In principle, if the kinetic model has two unknown parameters, at least two temperature histories are required, and if there are three unknown parameters, then at least three temperature histories are needed. The method was originally developed for nonlinear

microbial inactivation and chemical degradation reactions at very high temperatures and short times such as encountered in UHT preservation (M. Corradini, Normand, & Peleg, 2008; M.G. Corradini, Normand, Newcomer, Schaffner, & Peleg, 2009; Peleg et al., 2008). The initial assumption has been that the spores or survival cells follows Weibullian kinetics (Eq. 7) and that the temperature-dependence of the rate parameter, $b(T)$, follows the Log-Exponential model (ibid), which has a marker of the lethal temperature's onset. In contrast, the degradation of many nutrients and pigments in foods follows linear fixed order kinetics, which in many cases can facilitate the calculation (Peleg et al., 2014). When a nutrient is lost during storage, as already mentioned, the roles of the come-up and cooling times, or vice versa, are rarely if ever an issue and hence the endpoints method's advantage in this case is primarily logistic. Recent works showed that the endpoints method could be used to estimate vitamins degradation kinetic parameters, including the reaction's kinetic order if unknown, from isothermal or non-isothermal data (Peleg, Kim, & Normand, 2015; Peleg et al., 2014; Peleg & Normand, 2015). The endpoints method's validation came from its ability to predict correctly residual concentrations at temperature histories not used in the kinetic parameters determination. The method is considerably simplified when the reaction's kinetic order is known a priori or can be assumed based on reports in the literature (ibid). It is further simplified when the kinetic order is known and all the storage temperatures are constant, see next section.

2.5.3. The isothermal case

Consider a scenario where there are two food samples having a known initial concentration of ascorbic acid stored at two constant temperatures T_1 and T_2 for times t_1 and t_2, respectively, which resulted in their having corresponding concentration ratios C_1

and C_2. When the heating and cooling times are negligible relative to the storage duration, the temperature profile can be considered isothermal for all practical purposes. There are reports in the literature which suggest that the AA's degradation follows first order kinetics and we will address this case first. Scenarios where it does not will be discussed separately later.

Fixed order degradation kinetics, as already stated, follows Eq. 1 as a model and its isothermal solutions for $n = 1$ and $n \neq 1$ are Eq. 2 or 3, respectively. We assume that the temperature-dependence of the rate constant $k(T)$ defined by these equations follows the Arrhenius equation and hence the simpler exponential model (Eq. 9) too. When the exponential model holds, one can insert $k(T)$ as described by Eq. 9 into Eq. 2 or 3 to produce an algebraic isothermal degradation model for any temperature T in a pertinent range. [One can also replace $k(T)$ with $k[T(t)]$ and T by $T(t)$ and insert them into Eq. 1 to produce a general kinetic model of which isothermal degradation is a special case (Peleg et al., 2015).]

As shown in Figure 2.6 - Left, the two endpoints, $\{t_1,C_1\}$ and $\{t_2,C_2\}$, ought to be on two yet unknown degradation curves of their respective temperatures T_1, and T_2. To reconstruct these curves, see Figure 2.6 - Right, we start by picking an arbitrary reference temperature T_{ref}, preferably between or in the neighborhood of T_1 or T_2.

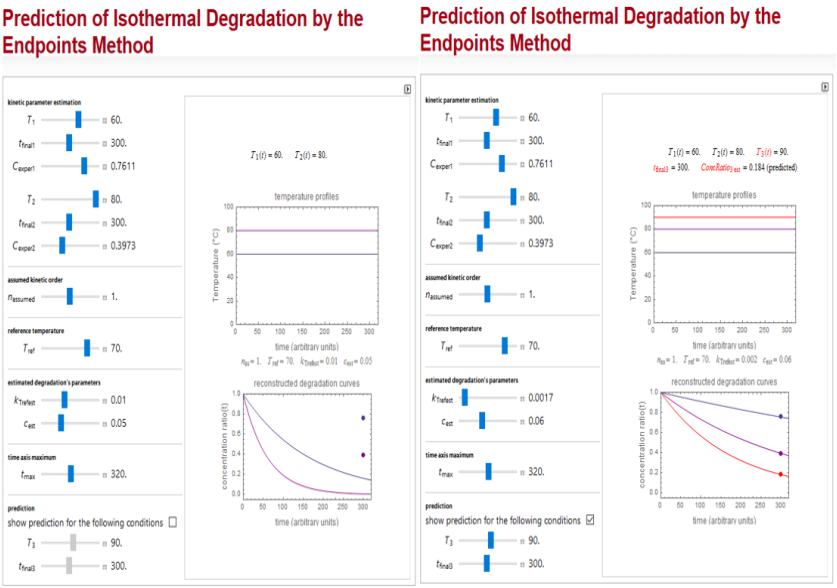


Figure 2.6. Screen displays of the Wolfram Demonstration that extracts the degradation kinetic parameters by the isothermal version of the endpoints method in the default and prediction modes: Left –The two endpoints before being matched by the reconstructed degradation curves. Right – The matched reconstructed curves obtained by moving the k_{Tref} and c_{est} sliders and a predicted degradation curve at a third temperature T_3 not used in the parameters calculation (marked in red). Notice the positions of T_3 and $t_{\mathrm{final},3}$ sliders.

If the degradation indeed follows first order kinetics and the rate constant temperature-dependence exponential model as assumed, then insertion of Eq. 9 into Eq. 2 implies that:

$$C_1 = \mathrm{Exp}[-k(T_{\mathrm{ref}})\ \mathrm{Exp}[c(T_1-T_{\mathrm{ref}})]\ t_1] \qquad (16)$$

and

$$C_2 = \mathrm{Exp}[-k(T_{\mathrm{ref}})\ \mathrm{Exp}[c(T_2-T_{\mathrm{ref}})]\ t_2] \qquad (17)$$

We can do the same with Eq. 3 if we know or want to try $n \neq 1$.

Eqs. 16 and 17 are two simultaneous nonlinear algebraic equations with two unknowns, namely $k(T_{\mathrm{ref}})$ and c. The two equations can be solved numerically with the FindRoot function of Mathematica® (Wolfram Research, Champaign IL), which is the program used to test the concept for ascorbic acid in this article and to extract the numerical values of

these two unknown parameters. This can also be done with similar equation solving functions of other commercial mathematical software.

Once $k(T_{ref})$ and c have been calculated in this way, they can be inserted back into the isothermal degradation model equation to reconstruct the two degradation curves shown in the figure, and to predict and plot the degradation curve at any other storage temperature T_3 in a pertinent range.

The validity of the endpoints method and its underlying assumptions can be tested by comparing experimental concentration ratios *at temperatures not used in the parameters calculation* with those predicted by the described procedure. An agreement between the experimental and predicted concentration ratios, especially when observed at several temperatures, will validate the method. Failure to render close predictions can have several interpretations. It can be due to an experimental error or errors in the data and/or to that one or more of the underlying assumptions are invalid. Examples are that the degradation in the particular food follows nonlinear kinetics, or a fixed kinetic order that is substantially different from that assumed. In extreme cases, violation of the assumptions may result in failure of the iterations to converge, or if they do converge, the rendered parameter values can be unrealistic or absurd.

It ought to be stated that two endpoints are the *smallest theoretical number* of temperatures, times, and corresponding final concentration ratios, which are needed to extract the values of the parameters $k(T_{ref})$ and c when the kinetic order n is known a priori. This should not be confused with the *number of measurements or chemical analyses*. These should always include several replicates to assure that the method renders reliable parameters values and make correct predictions. Also, wherever feasible, one should

determine and use more than two experimental endpoints, the theoretical minimal number, at least three. This will enable to validate the method and increase its predictions accuracy by averaging, see below.

To solve simultaneous nonlinear equations numerically requires close initial guesses of the sought kinetic parameters, which can be a daunting task. The development of an interactive version of the calculation procedure has eliminated this problem (Peleg, Normand, & Goulette, 2016). The program is available in the form of two Wolfram Demonstrations one for temperature above the freezing mark (http://demonstrations.wolfram.com/PredictionOfIsothermalDegradationByTheEndpointsMethod/) and the other for temperatures below (http://demonstrations.wolfram.com/EndpointsMethodForPredictingChemicalDegradationInFrozenFoods/).

2.5.4. Averaging the kinetic parameters obtained by the endpoints method

Suppose there are three experimental endpoints available for analysis, i.e., T_1, t_1 & C_1, T_2, t_2 & C_2, and T_3, t_3 & C_3, which we can call A, B and C for convenience. Applying the method to the three pair combinations A&B, A&C and B&C will render three values of $k(T_{ref})$: $k(T_{ref})_{A\&B}$, $k(T_{ref})_{A\&C}$, and $k(T_{ref})_{B\&C}$, and three values of c: $c_{A\&B}$, $c_{A\&C}$, and $c_{B\&C}$. These $k(T_{ref})$'s and c's values can be averaged to improve the parameters' reliability and consequently the quality of any fourth concentration ratio, c_4, at a temperature T_4, different from T_1, T_2, and T_3, which had been used in the parameters calculation. With four experimental endpoints available, the number of pair combinations for averaging is six, i.e., A&B, A&C, A&D, B&C, B&D, and C&D, and with five the number rises to ten, i.e., A&B, B&C, B&D, B&E, B&C B&D, B&E, C&D, C&,E and D&E, which can boost the

kinetic parameters' reliability dramatically, albeit at an added logistic cost. Also, when there are six or more values, one can identify outliers by statistical criteria and eliminate them from the average calculations (M. Corradini et al., 2008). In what follows in the next sections we identified suspected outliers by the two sided Iglewicz and Hoag test ("Detection of Outliers," n.d.) with z = 3.5 using a free online program (http://contchart.com/outliers.aspx).

2.6. Testing the isothermal version of the endpoints method with published ascorbic acid degradation data

2.6.1. The interchangeability of the Arrhenius and Exponential models in ascorbic acid degradation

The examples of reported $k(T)$ vs. T data of AA fitted with both the Arrhenius and exponential models given in Figure 2.3 are in agreement with previous observations in different nutrients and other chemical systems (Peleg et al., 2015, 2012, 2014). They demonstrate that the two models are indeed interchangeable at temperatures that are relevant to food processing and storage. The examples also demonstrate that as long as the reference temperature is in a pertinent range, its choice has no discernible effect on the two models' fit as expected.

2.6.2. Comparison of the endpoints' method predictions with reported data

As already explained, not all the available published data on ascorbic acid degradation are suitable for testing the applicability of the described version of the endpoints method, which was developed exclusively for degradation patterns that follow conventional first order kinetics. Since many of the original publications that we have surveyed reported the entire isothermal degradation dataset at different temperatures had a first order kinetic model's fit, we could identify several which were suitable for testing the

method. Examples of Wolfram's Demonstration's screen displays that were used to calculate the AA's degradation kinetic parameters from endpoints extracted from published isothermal data and to predict the concentration ratios at a third temperature are shown in Figures 2.7-2.11.

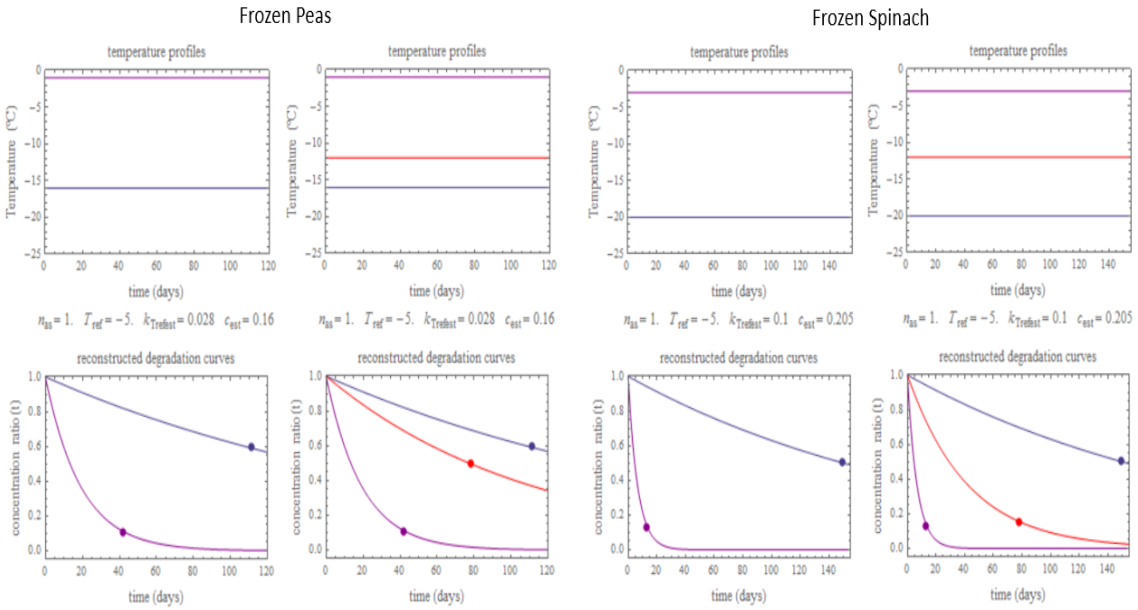


Figure 2.7. Left - the endpoints method applied to ascorbic acid loss in frozen peas. The reconstructed curves are in blue and purple, and the predicted is in red. The numerical values of the retrieved parameters and predicted retentions are listed in Tables 1 and 2. The experimental data are from (Giannakourou & Taoukis, 2003).

Figure 2.8. Right - the endpoints method applied method to ascorbic acid loss in frozen spinach. The reconstructed curves are in blue and purple, and the predicted is in red. The numerical values of the retrieved parameters and predicted retentions are listed in Tables 1 and 2. The experimental data are from (Giannakourou & Taoukis, 2003).

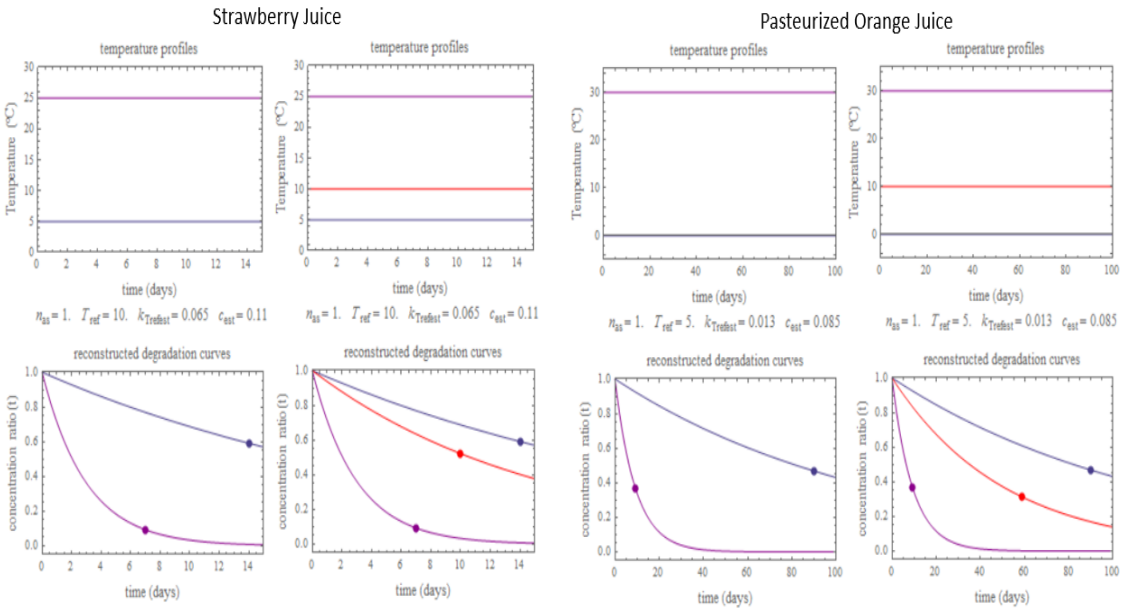


Figure 2.9. Left - the endpoints method applied method to ascorbic acid loss in strawberry juice. The reconstructed curves are in blue and purple, and the predicted is in red. The numerical values of the retrieved parameters and predicted retentions are listed in Tables 3 and 4. The experimental data are from (Derossi et al., 2010).

Figure 2.10. Right - the endpoints method applied method to ascorbic acid loss in pasteurized orange juice. The reconstructed curves are in blue and purple, and the predicted is in red. The numerical values of the retrieved parameters and predicted retentions are listed in Tables 3 and 4. The experimental data are from (Polydera et al., 2003).

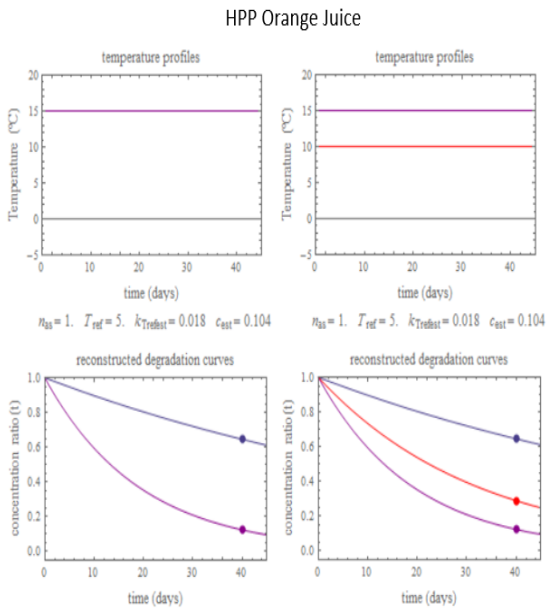


Figure 2.11. The endpoints method applied method to ascorbic acid loss in ultra high-pressure treated orange juice. The reconstructed curves are in blue and purple, and the predicted is in red. The numerical values of the retrieved parameters and predicted retentions are listed in Tables 3 and 4. The experimental data are from (Polydera et al., 2005).

The figures show the reconstructed degradation curves passing through the two entered endpoints (left), and the predicted degradation curve at a third temperature (right), using the $k(T_{ref})$ and c values obtained by matching the reported experimental endpoints with the generated (reconstructed) curves. In the right plot, the two reconstructed curves are plotted in blue and purple and the predicted curve in red.

Tables 2.1 and 2.3 summarize the endpoint combinations used to determine the AA's degradation kinetic parameters in frozen peas and spinach, and in strawberry and orange juices stored at constant ambient temperatures. Table 2.1 shows that with only a few exceptions, where outliers were suspected and consequently removed. The magnitude of the calculated $k(T_{ref})$'s and c's did not vary by much as a result of choosing different endpoints combinations. The table also shows that the suspected outliers removal did not have a dramatic effect on these kinetic parameters magnitudes.

Table 2.1. Kinetic degradation parameters of vitamin C in two frozen vegetables[1].

Food	T_1 (°C)	t_1 (days)	T_2 (°C)	t_2 (days)	Pair	k_{Tref} (t^{-1})	c (T^{-1})
Frozen Peas	-16	111	-12	94	AB	0.022	0.140
	-16	111	-8	104	AC	0.027	0.160
	-16	111	-3	80	AD	0.019	0.127
	-16	111	-1	42	AE	0.028	0.160
	-12	94	-12	94	BC	0.028	0.172
	-12	94	-3	80	BD	0.020	0.120
	-12	94	-1	42	BE	0.027	0.168
	-8	104	-3	80	CD	0.021	0.070*
	-8	104	-1	42	CE	0.027	0.160
	-3	80	-1	42	DE	0.012	0.380*
	T_{ref} = -5 °C				Mean ± SD	0.023 ± 0.005	0.166 ± 0.081
					Revised Mean ± SD	0.025 ± 0.004	0.151 ± 0.019
Frozen Spinach	-20	149	-12	78	AB	0.100	0.205
	-20	149	-8	41	AC	0.082*	0.192
	-20	149	-3	13	AD	0.100	0.205
	-12	78	-8	41	BC	0.080*	0.170
	-12	78	-3	13	BD	0.100	0.198
	-8	41	-3	13	CD	0.098	0.240*
	T_{ref} = -5 °C				Mean ± SD	0.093 ± 0.010	0.202 ± 0.023
					Revised Mean ± SD	0.100 ± 0.000	0.203 ± 0.004

[1] The original data are from (Giannakourou & Taoukis, 2003).
* Suspected outliers identified by the Iglewicz and Hoag test.

Table 2.2. Comparison of concentration ratios of vitamin C in two frozen vegetables predicted with the isothermal version of the endpoints method and those reported[1].

							% Retention	
Food	**Predicting**	**T_{ref} (°C)**	**k_{Tref}/Mean k_{Tref}**	**c/Mean c**	**T (°C)**	**t (days)**	**Predicted**	**Reported**
Frozen Peas	A	-5	0.025	0.155	-16	111	60	60
	B		0.025	0.152	-12	94	44	46
	C		0.023	0.143	-8	104	21	18
	D		0.026	0.160	-3	80	5	14
	E		0.023	0.144	-1	42	18	11
Frozen Spinach	A	-5	0.099	0.219	-20	149	57	50
	B		0.100	0.205	-12	78	16	15
	C		0.100	0.203	-8	41	11	14
	D		0.100	0.205	-3	13	14	13

[1] The original data are from (Giannakourou & Taoukis, 2003).

Table 2.3. Kinetic degradation parameters of vitamin C in stored fruit juices.

Food	T_1 (°C)	t_1 (days)	T_2 (°C)	t_2 (days)	Pair	k_{Tref} (t^{-1})	c (T^{-1})
Strawberry Juice[1]	5	14	10	10	AB	0.060	0.093
	5	14	25	7	AC	0.065	0.110
	10	10	25	7	BC	0.060	0.115
	T_{ref} = 10 °C				Mean ± SD	0.062 ± 0.003	0.106 ± 0.012
Pressurized Orange Juice[2]	65	385[a]	70	354[a]	AB	0.004	0.080
	65	385[a]	80	290[a]	AC	0.004	0.084
	70	354[a]	80	290[a]	BC	0.004	0.084
	T_{ref} = 70 °C				Mean ± SD	0.004 ± 0.000	0.083 ± 0.002
HPP Orange Juice[3]	0	40	5	40	AB	0.015	0.060
	0	40	10	40	AC	0.016	0.075
	0	40	15	40	AD	0.018	0.104
	5	40	10	40	BC	0.015	0.088
	5	40	15	40	BD	0.018	0.106
	10	40	15	40	CD	0.010	0.165
	T_{ref} = 5 °C				Mean ± SD	0.015 ± 0.003	0.100 ± 0.036
Pasteurized Orange Juice[3]	0	40	5	40	AB	0.021	0.040
	0	40	10	40	AC	0.021	0.034
	0	40	15	40	AD	0.025*	0.073
	5	40	10	40	BC	0.021	0.036
	5	40	15	40	BD	0.021	0.093
	10	40	15	40	CD	0.011*	0.150
	T_{ref} = 5 °C				Mean & SD (±)	0.020 ± 0.004	0.071 ± 0.045
					Revised Mean ± SD	0.0210 ± .0.000	0.051 ± 0.028
HPP Orange Juice[4]	0	109	5	91	AB	0.006	0.047
	0	109	10	64	AC	0.006	0.067
	0	109	15	46	AD	0.001*	0.118
	0	109	30	15	AE	0.007	0.090
	5	91	10	64	BC	0.006	0.086
	5	91	15	46	BD	0.006	0.150
	5	91	30	15	BE	0.006	0.098
	10	64	15	46	CD	0.003*	0.220
	10	64	30	15	CE	0.005	0.100
	15	46	30	15	DE	0.014*	0.062
	T_{ref} = 5 °C				Mean ± SD	0.006 ± 0.003	0.104 ± 0.050
					Revised Mean ± SD	0.006 ± 0.001	0.091 ± 0.032
Pasteurized Orange Juice[4]	0	90	5	90	AB	0.013	0.088
	0	90	10	59	AC	0.013	0.086
	0	90	15	41	AD	0.013	0.081
	0	90	30	9	AE	0.013	0.085
	5	90	10	59	BC	0.013	0.085
	5	90	15	41	BD	0.013	0.079
	5	90	30	9	BE	0.013	0.085
	10	59	15	41	CD	0.015*	0.066*
	10	59	30	9	CE	0.013	0.085
	15	41	30	9	DE	0.012*	0.088
	T_{ref} = 5 °C				Mean ± SD	0.013 ± 0.001	0.083 ± 0.007
					Revised Mean ± SD	0.013 ± 0.000	0.084 ± 0.003

[1] The original data are from (Derossi et al., 2010).
[2] The original data are from (Van den Broeck et al., 1998).
[3] The original data are from (Polydera et al., 2003).
[4] The original data are from (Polydera et al., 2005).
[a] time is in minutes
* Suspected outliers identified by the Iglewicz and Hoag test.

Table 2.4. Comparison of concentration ratios of vitamin C in stored fruit juices predicted with the isothermal version of the endpoints method and those reported.

							% Retention	
Food	**Predicting**	**T_{ref} (°C)**	**k_{Tref}/Mean k_{Tref}**	**c/Mean c**	**T (°C)**	**t (days)**	**Predicted**	**Reported**
Strawberry Juice[1]	A	10	0.060	0.115	5	14	62	59
	B		0.065	0.110	10	10	52	55
	C		0.060	0.093	25	7	18	9
Pressurized Oranges[2]	A	70	0.004	0.084	65	385[a]	34	33
	B		0.004	0.084	70	354[a]	22	22
	C		0.004	0.080	80	290[a]	7	6
HPP Orange Juice[3]	A	5	0.014	0.120	0	40	73	65
	B		0.015	0.115	5	40	55	56
	C		0.017	0.090	10	40	34	40
	D		0.015	0.074	15	40	28	12
Pasteurized Orange Juice[3]	A	5	0.021	0.065	0	40	54	50
	B		0.021	0.034	5	40	44	43
	C		0.021	0.067	10	40	31	37
	D		0.021	0.037	15	40	30	12
HPP Orange Juice[4]	A	5	0.006	0.109	0	109	70	62
	B		0.006	0.086	5	91	57	60
	C		0.006	0.096	10	64	54	57
	D		0.006	0.081	15	46	54	30
	E		0.006	0.088	30	15	45	37
Pasteurized Orange Juice[4]	A	5	0.013	0.081	0	90	46	46
	B		0.013	0.082	5	90	31	31
	C		0.013	0.084	10	59	31	31
	D		0.013	0.086	15	41	29	30
	E		0.013	0.081	30	9	37	37

[1] The original data are from (Derossi et al., 2010).
[2] The original data are from (Van den Broeck et al., 1998).
[3] The original data are from (Polydera et al., 2003).
[4] The original data are from (Polydera et al., 2005).
[a] time is in minutes
* Suspected outliers identified by the Iglewicz and Hoag test.

Tables 2.3 shows no suspected outliers and here too the $k(T_{ref})$'s and c's calculated with the different endpoint combinations also did not vary dramatically. Considering that in none of the original studies from which the data were obtained had given the endpoints any special consideration, i.e., the concentrations were determined with only 2 or 3

replicates, this constancy indicates that the method is fairly robust and that the assumptions on which it is based are not unrealistic.

The crucial test of the endpoints method applicability to ascorbic acid, however, is whether and how its predicted concentration ratios agreed with those reported in the original publications. Tables 2.2 and 2.4 list the predicted and reported values for comparison. In the case of the two frozen vegetables, see table 2.2, the agreement was consistently reasonable at least as judged by informal criteria, i.e., the difference between the predicted and reported percent retention is mostly on the order of 1-3% (absolute), with three notable exceptions where the discrepancies were on the order of 7-9% (absolute). As shown in Table 2.4, the agreement between the predicted and reported values in the stored juices was inconsistent. In most trials the discrepancies varied between fairly small, i.e., absolute difference of 0-5% in the retention level, and substantial that is as high as 9-12% (absolute). Since in none of the original studies from which the data were obtained had the endpoints received special attention, as already stated, the magnitude of discrepancies suggest that the endpoints method could have been more robust had special effort been made to determine the last concentration ratios more accurately. We suspect that the shown discrepancies were most probably, or at least partly, due to the quality of the individual data points and not to a systemic failure of the method. Had this been the case, it would be very difficult to explain why none of discrepancies was of an order of magnitude and why they showed no discernible trend or pattern. Also, all the original publications from which the data shown in the tables were extracted gave no indication of asymptotic approach to a residual, i.e., nonzero, retention level. Consequently, it is very unlikely that the discrepancies were due to an inappropriate model. Because the endpoints received no

special attention in the original studies, the occasional observed discrepancies and their magnitudes should have been expected rather than come as a surprise. All this re-emphasizes that if and when the endpoints method is implemented in storage studies, the endpoints concentration ratios should always be determined in a sufficient number of replicates.

2.6.3. Potential applications of the endpoints method with non-isothermal data

Consider two temperature profiles $T_1(t)$ and $T_2(t)$, at least one of them not isothermal, with corresponding endpoint concentration ratios C_1 and C_2, respectively. We assume that the degradation follows known fixed order kinetics, and that the rate constant's temperature-dependence obeys by the exponential model (or Arrhenius equation). If so, then the two endpoint concentration ratios C_1 and C_2 are the solutions of Eq. 11 for the two temperature profiles $T_1(t)$ and $T_2(t)$ for times t_1 and t_2, respectively. Or mathematically:

$$The\ solution\ for\ t_1\ of\ \frac{\mathrm{d}C(t)}{\mathrm{d}t} = -k\left(T_{ref}\right)Exp\left[c\left(T_1(t) - T_{ref}\right)\right]C(t)^n = C_1 \qquad (18)$$

$$The\ solution\ for\ t_2\ of\ \frac{\mathrm{d}C(t)}{\mathrm{d}t} = -k\left(T_{ref}\right)Exp\left[c\left(T_2(t) - T_{ref}\right)\right]C(t)^n = C_2 \qquad (19)$$

These two simultaneous equations can be solved numerically with Mathematica® to extract the values of the two unknown $k(T_{ref})$ and c. Once obtained, these parameters values can be used to reconstruct the entire degradation curves of the two temperature profiles $T_1(t)$ and $T_2(t)$ and predict concentration ratios at different times along them or at different temperature profiles to test the method. The concept and calculation procedure have been validated with computer simulations and published data on anthocyanins degradation (Peleg et al., 2015) but are yet to be tested with AA data. A freely downloadable interactive Mathematica® program that demonstrates the concept and calculation procedure method can be found at

http://people.umass.edu/aew2000/nutrient_degradation/InterpolatedDegradation.html.

The program solves the two simultaneous equations by moving the $k(T_{ref})$ and c sliders on the screen until the two reconstructed degradation curves at $T_1(t)$ and $T_2(t)$ pass through their corresponding endpoints $\{t_1,C_1\}$ and $\{t_2,C_2\}$. The program has one version particularly suitable for heat processing temperature and another for storage temperatures. Also, both versions offer the options to enter the two temperature profiles $T_1(t)$ and $T_2(t)$ in the form of algebraic expressions or digitized time-temperature files, which it automatically converts into smooth interpolation functions for use in the parameters calculation.

2.6.4. The successive points method

In principle, the parameters of a degradation reaction following a known kinetic order can be extracted from successive concentration ratios determined during a *single non-isothermal temperature history.* The method is based on a special case of Eqs. 18 and 19 where $T_1(t) = T_2(t) = T(t)$. Hence, for first or other fixed order kinetics degradation where the rate constant's temperature-dependence follows the exponential model, $k(T_{ref})$ and c are the numerical solutions of the two simultaneous equations (Peleg & Normand, 2015):

$$The\ solution\ for\ t_1\ of\ \frac{\mathrm{d}C(t)}{\mathrm{d}t} = -k(T_{ref})Exp\left[c(T(t) - T_{ref})\right]C(t)^n = C_1 \qquad (20)$$

$$The\ solution\ for\ t_2\ of\ \frac{\mathrm{d}C(t)}{\mathrm{d}t} = -k(T_{ref})Exp\left[c(T(t) - T_{ref})\right]C(t)^n = C_2 \qquad (21)$$

A freely downloadable interactive program that demonstrates the successive method can found at

http://demonstrations.wolfram.com/DegradationParametersFromConcentrationRatios/.

This version of the program allows the user to use any entered temperature profile equation or actual digitized time-temperature data can be found at http://people.umass.edu/aew2000/nutrient_degradation/NutrientDegradation.html. In both versions, the program finds a numerical solution to the two equations by moving the $k(T_{ref})$ and c sliders until the (single) reconstructed degradation curve passes through the two entered (experimental) points $\{t_1,C_1\}$ and $\{t_2,C_2\}$. The second and more elaborate version of the program also offers the option to make predictions, which can be tested against entered experimental data (Programs A to C). The successive points method has been tested with computers simulations and published data on vitamin A. It might as well apply to AA degradation, but only in scenarios where it follows first or other fixed order kinetics.

2.6.5. Non-linear kinetics

When the AA's isothermal degradation approaches an asymptotic residual retention level (Eq. 5) or follows the Weibullian model, its kinetics is defined by *three* kinetic parameters instead of two. In principle, the endpoints and successive points methods can be used to extract these models' parameters by numerically solving three instead of two simultaneous equations. Indeed, this can and has been done with simulated data that had no or very small scatter. Increasing the scatter to levels encountered in experimental concentration measurements almost invariably results in failure of the iterations to converge or unrealistic and frequently absurd parameter values (e.g., negative or complex numbers). The problem can be circumvented by solving only two equations iteratively with one of the sought parameters rising by small increments or falling by small decrements. At each step, the calculated intermediate parameters are used to predict the third endpoint

concentration and the iterations stop when the discrepancy between the predicted and actual concentration ratios becomes smaller than the user's specified tolerance (Peleg et al., 2008). Such a program already exists for the Weibullian model (Eq. 7) for cases where the power m is unknown, assuming that it is practically temperature-independent. Since that model was written (and tested) for microbial inactivation, the temperature-dependence term is not the exponential model. If needed, the program could be easily modified to accommodate the exponential model. Obviously, where the exponent m is known or can be assumed, the need for the iterative procedure is eliminated. Thus assuming that $b(T)$ in Eq. 7 follows the exponential model, one can determine the kinetic parameters c and $k(T_{ref})$ by the non-isothermal version of the two endpoints method, that is by solving a pair of simultaneous equations numerically. A freely downloadable Mathematica® program that does it for Weibullian degradation can be found at http://people.umass.edu/aew2000/Weibullian_degradation/WeibullianDegradation.html. The main issue, however, would still be how to know in advance whether there is an asymptotic residual concentration in which case this program will not work – see below.

2.7. Concluding remarks

The literature on ascorbic acid degradation during thermal processing and storage suggests the existence of at least three possible main patterns: conventional first order kinetics, initial exponential decay changing to an asymptotic approach to a residual retention level, and nonlinear kinetics, e.g., Weibullian, decay all the way. The three patterns might be practically indistinguishable initially, and in particularly slow degradation could even be indistinguishable from zero order kinetics. But if the corresponding models are used for extrapolation in order to predict the AA retention in

foods stored for long times, i.e., well beyond the experiment time scale, they could lead to very different results. In light of the inherent scatter in AA's concentration determinations in foods, it is unlikely that statistical considerations alone would be helpful to identify the degradation pattern unambiguously from short-term experimental data. It would therefore be a challenge to researchers to find *a chemical marker or markers*, if they exist, which would indicate whether the degradation tends to be complete (e.g., first order or Weibullian kinetics), or if it will end up with residual retention or a transition to a slower rate regime (e.g., the asymptotic residual or biphasic model). Although not discussed in this review, the roles of oxygen tension and perhaps catalysts presence might provide the key in certain foods.

Where applicable, the endpoints method's advantage over the traditional ways to estimate kinetic parameters from storage data is primarily logistic. It could eliminate the need to monitor the AA's concentration periodically resulting in significant saving. In addition, the two freely downloadable interactive Wolfram Demonstrations, for foods at ambient temperatures and cold or frozen storage, enable the extraction of the kinetic parameter in a matter of minutes, eliminating the need to plot the experimental data and/or subject them to linear or nonlinear regression. In thermal processing, the endpoints method could eliminate the problem of how to account for the come-up and cooling times' roles when withdrawing samples for the analysis.

In the endpoints method versions for which there is free software on the internet, the main underlying assumption is that the AA's degradation follows kinetic patterns that have been described in the literature. This assumption is testable. If wrong, then either the method would not work at all, i.e., no match between the endpoints and reconstructed

curves could be achieved, or its predictions would be consistently off mark. In the first order kinetic case, one could assume a different kinetic order and move the *n*-slider to a contemplated new *n* value. The two Wolfram Demonstrations allow the user to move the *n*-slider to the right or left, retrieve the new $k(T_{ref})$ and *c* values, recalculate the predicted concentration ratio, and compare it with the actually observed in a few minutes. Actually doing this revealed that with $n = 1.00 \pm 0.05$, the retrieved parameters and predicted retention values are only very slightly affected. In other words, the method seems to be robust against, or insensitive to slight deviations from the assumed first kinetic order, if indeed they are real.

The endpoints method, as already mentioned, was originally developed for UHT sterilization where the processing time is too short for retrieving samples for analysis. Such a process only allows to examine the product after its completion, which includes the cooling stage, and hence the method's name. In storage, if there is a suspicion that the AA's degradation might not follow the assumed kinetics, one can test samples *early during the storage* to confirm or refute the hypothesis. Obviously, this will add to the number of concentration measurements, but their total number will still be smaller than in systematic concentration determinations at fixed intervals. If the suspicion is confirmed, then one could test a different *n*, for example, or any of the available alternative models. In the worst case, one could always resort to the traditional method of recording the entire degradation curves and search for a new degradation kinetic model. In case where two or more of the presented models render similar predictions of the AA's retention, it would be prudent to use the one that predicts the lowest retention in order to be on the safe side from a nutritional viewpoint (Peleg, Normand, Dixon, & Goulette, 2016).

CHAPTER 3

CONTROLLING VITAMIN C LOSS DURING RETORT THERMAL PRESERVATION

3.1. Abstract

Vitamin content and degradation are major concerns regarding space food. Throughout their production, vitamins are lost due to unavoidable environmental conditions meant to extend the food shelf life and maximize food safety. However, monitoring vitamin C loss periodically during routine thermal processing of solid foods is technically unfeasible. To understand the relationship between vitamin degradation, time, temperature, and to predict/control vitamin loss during thermal processing, a model was developed that can derive the degradation parameters, which describe the degradation behavior of a given compound in a given food matrix, from merely the vitamin concentration endpoints and time-temperature records of two thermal processes. In this study, two NASA-utilized space foods: rhubarb applesauce and sugar snap peas were heat stabilized using three thermal processes. Time-temperature records were given throughout each process, and vitamin C content was determined before and after each process. Rhubarb applesauce represented a low pH ($pH < 4.6$) food matrix and sugar snap peas represented a high pH ($pH \geq 4.6$) product. All foods were prepared per NASA instruction and packaged with nearly identical packaging material as used by NASA products. After analyzing vitamin C concentration before and after thermal processing and thereby determining the kinetic degradation parameters of each food, our model demonstrated less than 9% residual average difference between experimental and predicted concentration values showing a 2.7% difference for rhubarb applesauce and a 7.8% difference for sugar

snap peas. Overall, the model showed promising applications making vitamin C predictions to control vitamin C loss in foods exposed to thermal processing.

3.2. Introduction

Vitamin C (Vit C) is an essential vitamin that can be acquired through many foods or beverages. The loss of vit C can alter the stability of many food products. The stability of vit C in solid foods is influenced by many factors, such as processing method, packaging (e.g. metal cans, glass jars/bottles, flexible pouches, or rigid trays), food matrix, pH, oxygen, light, temperature, and pressure [e.g. ((Oey, Verlinde, Hendrickx, & Van Loey, 2006)]. Retort processing, a unit operation in which foods are heated at a sufficiently high temperature for a sufficiently long time to reduce microbial viability and enzyme activity to prolong food shelf life, is a common processing method for many shelf-stable foods. Although a crucial process for food safety, it can induce a significant loss in vit C due to the vitamin's vulnerability to elevated temperatures. On the contrary, high temperature-short time (HTST) retort processing has been associated with improving the quality and nutrient stability of foods in retort packaging (Hassan & Ramaswamy, 2013). Nonetheless temperature is still a large culprit of vit C stability, but time under heat must also be taken into consideration.

There are many theoretical models in thermal processing to describe the chemical and temperature dependence of chemical reactions. Simple chemical reactions are the most common approach used to describe the temperature dependence constant, k, reference from the Arrhenius equation:

$$k = Ae^{(-E_a/RT)} \qquad (1).$$

A is the frequency factor of the pre-exponential equation; E_a is the activation energy; R is the universal gas constant; and T is the absolute temperature. This is just one of the many forms of the Arrhenius equation (Boekel, 2008). Although this model is widely used, there are alternative models that can successfully describe temperature dependence of chemical reactions. The following paper will focus on the endpoints method model. The theory and underlying mathematics behind this model is similar to the "Prediction of Isothermal Degradation by the Endpoints Method" model (Peleg, Normand, & Goulette, 2016); however, this model incorporates non-isothermal data to determine degradation kinetics. The mathematics behind the model is described partially as a rate equation, which is referenced below:

$$\frac{\mathrm{d}C(t)}{\mathrm{d}t} = -k[T(t)]C(t)^n \qquad (2).$$

Under first order kinetics (n = 1) with a constant temperature T(t) = T and an initial concentration (C_0) at C(0) = 1, an algebraically solvable equation can be written:

$$\frac{\mathrm{C\ (t)}}{C_0} = e^{[-\mathrm{k(T(t))t}]} \qquad (3)$$

where C(t) is the momentary concentration of vit C at time (t); C(t) over C_0 represent the concentration ratio; k[T(t)] is the rate constant referenced at a set temperature and time; and n is the reaction order (Peleg, Normand, Dixon, & Goulette, 2016). Since many authors used first order kinetics for vit C degradation using a variety of food matrices and temperature profiles, the model example will be based on first order kinetics; however, the order can be manipulated with some adjustments to the equations [e.g. (Burdurlu, Koca, & Karadeniz, 2006; Cruz, Vieira, & Silva, 2008; Giannakourou & Taoukis, 2003; Polydera, Stoforos, & Taoukis, 2003; Van den Broeck, Ludikhuyze, Weemaes, Van Loey, & Hendrickx, 1998; Vieira, Teixeira, & Silva, 2000)].

Equation 4 gives the temperature rate constant at any time. This is the rate temperature dependence equation for determining degradation parameters:

$$k[T(t)] = k_{T_{ref}} e^{[c(T(t)-T_{ref})]} \qquad (4)$$

where t represents time (s); k_{Tref} is the rate constant at a set reference temperature (T_{ref}); T(t) is a time dependent temperature variable; and c is a temperature sensitivity constant, related to activation energy in the Arrhenius equation. When the temperature-dependence of the rate constant coincides with the exponential equation, equation 4 can be placed into equation 3 to create equation 4:

$$\frac{C(t)}{C_0} = e^{-k_{Tref} * e^{\left[c*\left(T(t)-T_{ref}\right)\right]*t}} \qquad (5)$$

Once c and k_{Tref} are known, you can use equation 4 to determine the predicted concentration ratio for isothermal data. To make this model applicable for non-isothermal predictions, an interpolated function of the time-temperature heat profile data is made in Wolfram Mathematica and ordinary differential equations 6 and 7 can be numerically solved simultaneously with Mathematica NDSolve function while using the slider feature in the program's interface to estimate unknown degradation parameters: k_{Tref} and c

$$\frac{dC(t)}{dt} = -k_{T_{ref}} * e^{[c*(T_1(t_1)-T_{ref})]} * C_1(t)^n \qquad (6)$$

$$\frac{dC(t)}{dt} = -k_{T_{ref}} * e^{[c*(T_2(t_2)-T_{ref})]} * C_2(t)^n \qquad (7)$$

with the boundary condition $C(0) = 1$ for both.

The estimated degradation parameters can be used to build a degradation curve that can predict the concentration ratio at any reasonable time and temperature profile. For example, profiles $T_1(t_1)$ and $T_2(t_2)$, k_{Tref}, and c solutions can determine the degradation curve for an arbitrary $T_x(t_x)$ (i.e. x = 1, 2….x-1) time-temperature profile. The theory

behind the model is further explained in this article (Peleg & Normand, 2015). An example of the model with rhubarb applesauce will be explained in the Materials and Methods section. Overall, the study has three objectives. First, to determine vit C degradation during thermal processing of rhubarb applesauce, high-acid food products (pH < 4.6) and sugar snap peas, a low-acid food product (pH $\geq$ 4.6). Secondly, use the experimental data to validate the nonisothermal endpoints method model vit C concentration predictions with other known temperature profiles that was not included in the experimental data used to attain degradation parameters. Lastly, we want to explore the impact of using different fixed order kinetics and reference temperatures. We hypothesized that using the nonisothermal endpoints method model with fixed first order kinetics will be a resourceful tool to reduce samples needed during thermal processing degradation studies and theoretically determine vit C degradation during thermal processing. Additionally, the reference temperature fluctuation will have little to no impact on predictions with subtle adjustments.

3.3. Materials and Methods

3.3.1. Analytical material

Vitamin C (Vit C) standards were referenced from L-Ascorbic Acid (99% purity) purchased from Fisher Scientific. Vit C food extraction utilized three extraction stabilizers: TCEP hydrochloride (reducing agent) purchased from Thermo Scientific, ethylenediaminetetraacetic acid (EDTA) disodium salt dehydrate (chelator) purchased from Sigma-Aldrich, metaphosphoric acid (MPA; pH reducer; 33.3-36.5% HPO_3 purity) purchased from Reagent World, Inc., and distilled laboratory water. The mobile phase (MP) for HPLC analysis consisted of 4 reagents: EDTA (99% purity) purchased from

Acros Organics, sodium acetate (pH 3.0±0.1) purchased from J.T. Baker Chemical Co., dodecyltrimethylammonium bromide (DTAB; 99% purity), phosphoric acid (pH adjuster; 85-90% purity) purchased from Fluka Analytical, and triple deionized laboratory water.

3.3.2. Food material

Stringless sugar snap peas (Mann Packing Co., Inc.), frozen rhubarb, unsweetened canned applesauce (West Creek), and sliced strawberries 4+1 (Simplot Classic) were purchased from Performance Food Service (One Performance Boulevard, P.O. Box 3024, Springfield, MA, USA). Sugar snap peas gravy ingredients: butter, noniodized salt, cornstarch, and ground black pepper were all purchased from local grocery store. Tap water was used to sugar snap pea make gravy.

3.3.3. Packaging

Retort pouches were opaque aluminized pouches with a thermal seal coating [12.065 cm x 20.48 cm (4.75" x 8.0625"); Tan PE/.0007Foil/3mil Coex Sealant] that were purchased from Heritage Packaging (441 Market St, Lawrence, MA 01843, USA). VacMaster SVP 20 (Overland Park, KS 66211, USA) at 1.016 bar (~30 in. Hg) was used to seal pouches. All specifications were similar to those used by NASA.

3.3.4. Rhubarb applesauce and sugar snap peas preparation

Rhubarb Applesauce contained three ingredients: unsweetened applesauce (40%; w/w), frozen diced rhubarb (40%; w/w), and frozen strawberries 4+1 (20%; w/w). Applesauce was manually mixed with blended strawberries and rhubarb was folded into mixture until uniform. Final product content was filled in retort pouches with a minimum fill weight of 142 g and a maximum fill weight of 156 g. Sugar snap peas 6.4 kg (~14 lbs.) were blanched in boiling water at 100 °C (212 °F) for 3 minutes in kettle and immediately

submerged in ice-cold water at 0 °C (32 °F) until cool. A starch slurry gravy was made to coat the sugar snap peas. Butter 0.22 kg (~0.5 lbs.) and 1,048 mL of water were melted and mixed in a large stainless-steel pot on medium high until content temperature was above 81.7 °C (170 °F). Salt (55 g), cornstarch (69 g), and black grounded pepper (7 g) were mixed in a separate bowl. After mixing, 135 mL ambient temperature tap water was added to mixture slowly. This mixture was mixed into the water/butter solution on the stove once it recorded over 81.7 °C (170 °F). The content on the stove was reheated to 81.7 °C (170 °F) and was held at 170 °F for 3-5 minutes. When the starch slurry gravy was completed, a Bostwick consistometer was used to verify a consistency of 16.5 cm $\pm$ 0.5 cm in 15 seconds at 81.7 °C (170 °F). Once the consistency parameters were met, the gravy was coated on the sugar snap peas. According to NASA specifications, sugar snap peas were filled into each retort pouch at a minimum weight of 100 g and a maximum weight of 114 g. Between 14 and 21 g of the starch slurry gravy was added to each retort pouch. Pouches were labelled, and final weights were recorded. All pouches from each food were vacuum sealed with VacMaster SVP 20.

3.3.5. Experimental design

To validate our thermal processing model, six retort processes or in industry speak, "recipes," were created using three distinct profiles of varying time duration and cook temperature targets for each food. Recipes were created in-house following industrial standard and NASA requirement of equivalent lethality or a minimum F_0 of 6 for sugar snap peas (a low acid food), and a minimum cook at 91.3 °C (200 °F) for 2 minutes for rhubarb applesauce (high acid foods). We adjusted the cook temperature and time to get a broad range of thermal processing for each food while ensuring lethality requirements were

met. See Table 3.1 to view all retort recipes for each food. All recipes were retorted using an Allpax 2402-R3 R&D horizontal batch retort (Allpax Products, LLC, 13510 Seymour Meyers Blvd., Covington, LA 70433 USA), by the preprogramed water spray method. Additionally, an HH378 Omega data logger (OMEGA Engineering, INC., 800 Connecticut Ave., Suite 5N01, Norwalk, CT 06854 USA) was used to track sample temperature data in 30 s intervals and Se379 software (Cetani Corporation, 11495 N Pennsylvania St Suite 240, Carmel, IN 46032 USA) recorded the time-temperature data on the computer in comma separated value format. The temperature experienced by the samples was recorded with four interspersed probes within the retort vessel to provide the non-isothermal temperature profile for the model. To gather readings, pouches were punctured on one side with a 10 mm (3/8") hole puncher by placing an 18 x 4 x 1.5 cm cedar wood block inside pouch and hammering down on hole puncture to open hole in pouch. A thermocouple consisting of 61 cm (2') of TEF-20 wire with a hot junction on one end and a C-7.1 female locking connector on the other end was used to track temperature. A C-5.2 stuffing box was used to secure hole while hot junction was adjusted to center of sample. Female locking unit was connected to the C-10 locking male connector, which provided a 2-line path to data logger. All material for thermocouple was purchased from Ecklund-Harrison Technologies Inc., 11000 Metro Pkwy, Ste. 40, Fort Myers, FL 33966-1245 USA.

Table 3.1. Retort recipes for the low and high acid temperature profiles

Low Acid Foods					High Acid Foods				
Food	Segment	Time (min)	Temp (°F)	Pressure (PSI)	Food	Segment	Time (min)	Temp (°F)	Pressure (PSI)
Sugar Snap Peas Low Temp/Short Time (A)	Come-Up Fill	-	180	5	Rhubarb Applesauce/Strawberries Low Temp/Short Time (A)	Come-Up Fill	-	115	5
	Come-Up	10	248	35		Come-Up	10	191	35
	Cook	17	244	35		Cook	8	190	35
	Pressure Cool	10	120	10		Pressure Cool	5	110	10
	Pressure Cool	5	110	5		Pressure Cool	5	100	5

	Atmospheric Cool	5	80	-		Atmospheric Cool	5	80	-
Sugar Snap Peas Mod Temp/Mod Time (B)	Come-Up Fill	-	180	5	Rhubarb Applesauce/Strawberries Mod Temp/Mod time (B)	Come-Up Fill	-	115	5
	Come-Up	10	255	35		Come-Up	5	213	35
	Cook	12	252	35		Cook	5	210	35
	Pressure Cool	10	120	10		Pressure Cool	5	110	10
	Pressure Cool	5	110	5		Pressure Cool	5	100	5
	Atmospheric Cool	5	80	-		Atmospheric Cool	5	80	-
Sugar Snap Peas High Temp/Short Time (C)	Come-Up Fill	-	180	5	Rhubarb Applesauce/Strawberries High Temp/Short Time (C)	Come-Up Fill	-	115	5
	Come-Up	10	255	35		Come-Up	2	225	35
	Cook	7	252	35		Cook	2	220	35
	Pressure Cool	10	120	10		Pressure Cool	5	110	10
	Pressure Cool	5	110	5		Pressure Cool	5	100	5
	Atmospheric Cool	5	80	-		Atmospheric Cool	5	80	-

3.3.6. HPLC analysis of vitamin C

Vitamin C (Vit C) content was determined experimentally by using a modified version of the AOAC Official Method 2012.21, "Vitamin C in Infant Formula and Adult/Pediatric Nutritional Formula with UV Detection" (Schimpf, Thompson, & Baugh, 2013) where vit C was detected using an HPLC Agilent Technologies 1100 series with a VWD detector (g1314A), degasser, and binary pump. Agilent OpenLab CDS ChemStation Edition was used to record data. The detector was set to 254 nm. A Synergi Polar-RP, 2.5 μm, 100 Å, 3 × 100 mm column from Phenomenex was used for separation. The flow rate was set to 0.4 mL/min, injection volume (20 μL), and a 15 min run time was used for analysis.

Preparation began with blending the entire retort pouch sample in a 250 mL stainless steel blender cup using a Waring blender on low intensity for at least 15 s. For sugar snap peas, blender container lids were sometimes opened and food mixture was manually agitated to ensure a smooth, homogenous blend during this process. Post-blending, 2 mL bead ruptor tubes (tubes designed for use with bead ruptor homogenizer) were filled with 4 (2.8 mm) ceramic beads (beads were specifically designed for bead

ruptor that maximized vit C physical extraction). Then, an aliquot of 230 mg and 180 mg for rhubarb applesauce and sugar snap peas, respectively, were added to a bead ruptor tube. Extraction reagents (EDTA disodium salt (5%), TCEP (0.1 %), 6% MPA (5%), and triple deionized water (89.9%) were added in a 1 mL proportion for rhubarb applesauce and 1.5 mL proportion for sugar snap peas to obtain proper dilution based on expected original vit C amount. Samples were blended with Omni bead ruptor 24 (Omni International, Inc., 935-C Cobb Place Blvd. NW Kennesaw, GA 30144 USA) homogenizer for 2 mins on max intensity. The specific program was set to S = 8.00 (max speed), T= 10 s (shake time), C = 3 (# of cycles), and D = 30 s (rest time). This cycle was used to maximize extraction efficiency while minimizing heat production. The samples were then centrifuged at 14,000 RPM for 15 mins at 4 °C to separate fibrous content. Prior to HPLC analysis, samples were filtered through a 13 mm, 0.45 μm nylon filter membrane. Standards of 7, 15, and 30 mg/L were used to construct a standard curve and convert reported intensity areas from HPLC to known vit C concentrations.

3.3.7. Data processing

The following experiment was conducted to study the effects of processing conditions on vit C degradation. We utilized a model related to the "Prediction of Isothermal Degradation by the Endpoints Method" that process non-isothermal data entries from retort processing. The vitamin content was measured at the beginning and end of each thermal process. The temperature profile and vitamin content information were used to solve for degradation parameters "k_{Tref}" and "c", which describe the rate of vitamin loss at a set reference temperature and the intrinsic temperature sensitivity of the vitamin, respectively. Three temperature profiles were obtained during three distinct retort

processes for each food. Pairwise temperature profiles were used to determine theoretical degradation parameters of vit C degradation during retort thermal processing. The model's reliability was determined by comparing the predicted concentration ratio with the experimental concentration ratio. Each temperature profile was categorized as A, B, or C where the process data representing a low temperature/long time motif was represented as A, moderate temperature/moderate time was represented as B, and high temperature/short time was represented as C. Moving forward the process will be reference as the food abbreviation (i.e. SP: sugar snap peas or RA: rhubarb applesauce) followed by the temperature profile (i.e. Low for A, Mod for B, or High for C). For example, rhubarb applesauce with a moderate temperature/moderate time (B) will be abbreviated as "RAMod" and same lexicon for sugar snap peas.

For example, Figure 3.1 in stage 1 top graph shows temperature profiles B and C used to process rhubarb applesauce. This information was imported directly into the Mathematica program from a .csv document containing the time (in seconds) and temperature data (in degrees Celsius) to delineate the interpolated Mathematica time-temperature curve. Times t_{final1} and t_{final2} are populated based on the endpoint of the imported time-temperature data and occurred at 29 minutes for profile B and 23 minutes for C. The corresponding C_{exper1} and C_{exper2} represent the concentration ratios ($C_x/C_{initial}$; x is the endpoint concentration of a given process). As stated before, many researchers support that vit C follows first order kinetics, so the kinetic order was set to one to begin with. The reference temperature was set to 80 °C, which was near the average cook temperature among the three retort runs. To determine the degradation parameters, the sliders denoting k_{Tref} and c were adjusted such that the degradation curves outputted by the

model were as close as possible to the center of the matching colored dots to solve our two unknown parameters: k_{Tref} and c.

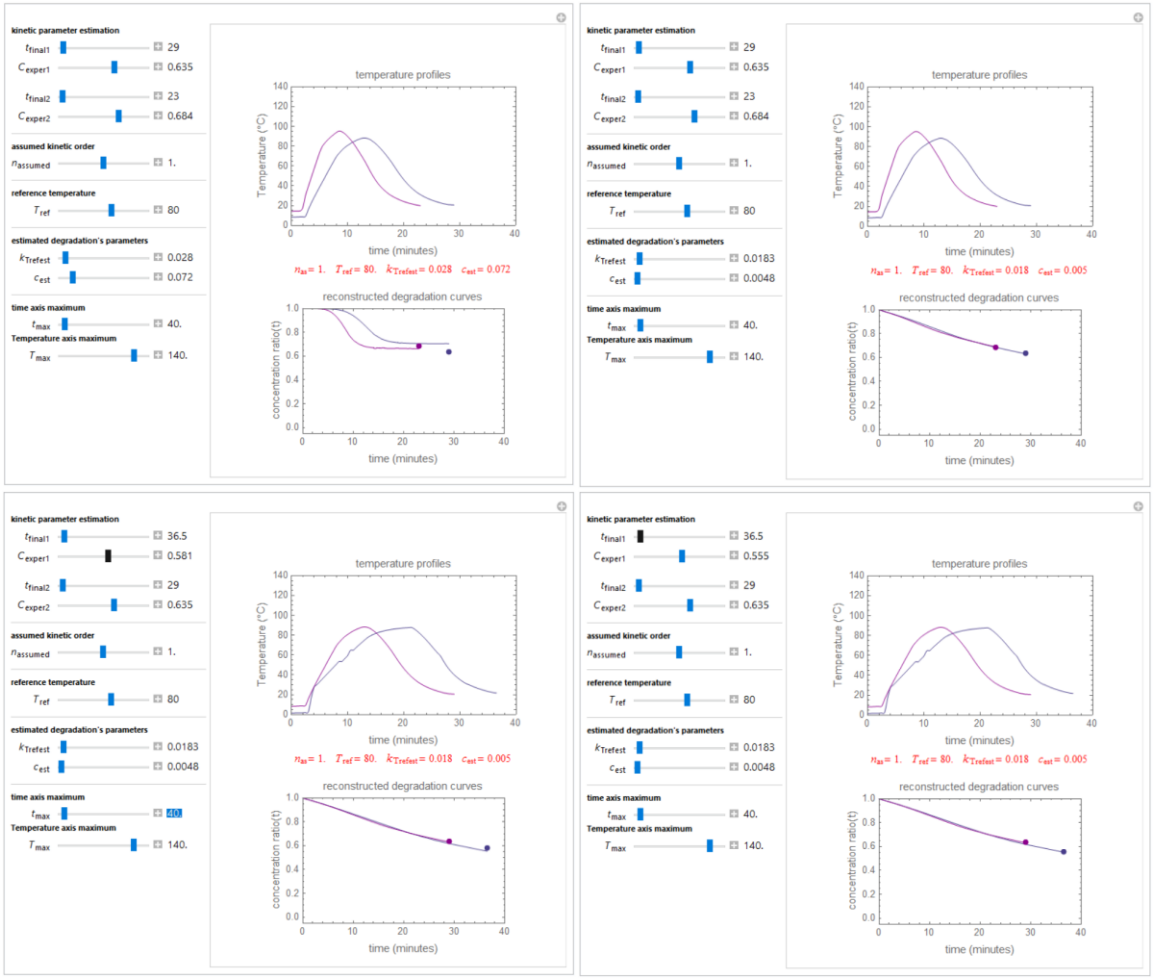


Figure 3.1. Stage 1: inputting BC known temperature profiles and vit C concentration ratios for rhubarb applesauce (RA); Stage 2: matching degradation curves with corresponding color concentration ratio dots to determine k_{Tref} and c; Stage 3: using AB temperature profiles and vit C concentration ratios to predict A's vit C degradation curve; and Stage 4: matching A's experimental concentration ratio with A's degradation curve to compare A's predicted concentration ratio with its experimental concentration ratio.

Figure 3.1 stage 2 demonstrates the closest match for k_{Tref} and c, therefore a suggestion of the actual degradation parameters of vit C during retort thermal processing. The bottom section shows additional slider tools to change the scale of the graph and improve the graph's data fitting. The degradation parameters from B and C temperature

profiles can be used to predict the vit C concentration for any temperature profile. To determine the kinetic parameters prediction accuracy, the predicted and measured vit C concentration ratios were compared. To do this, B or C temperature profile was replaced with A's temperature profile. In Figure 3.1 stage 3, we used B temperature profile and replaced it with A's temperature profile, and the concentration ratio was updated based on A's temperature profile. The estimated degradation parameters k_{Tref} and c were kept the same. To gauge prediction accuracy, the measured concentration ratio (shown by the blue dot) in Figure 3.1 stage 4 was adjusted by using the C_{exper1} slider until the blue degradation curve line was centered with the dot. The new concentration ratio for A temperature profile represents the estimated concentration ratio prediction.

This calculation method is freely accessible online by going to http://people.umass.edu/aew2000/ and clicking on the link "to estimate the kinetic degradation parameters of compounds in stored and thermally processed foods" and downloading the file: DegradationParametersEstimationFromInterpolatedTemperatureB(HeatProcessing).cdf. The cdf player can be downloaded on the wolfram demonstrations website. The model is also operable and editable in Wolfram Mathematica (Peleg, 2017).

3.3.8. Statistical analysis

All Vit C concentrations were expressed as mean $\pm$ standard deviation (SD) across the six replicates for each food and thermal process. Residuals were used to determine the differences between experimental and predictive data. The coefficient of variation and a 0.95% confidence interval was used to compute sample size and minimize relative error.

3.4. Results and discussion

3.4.1. Retort temperature profiles

Figure 3.2 provides a representation of all the retort temperature profiles for each food where four interspersed retort pouches were probed for each load and the average temperature was taken to represent the overall temperature profile. Commercially, lowest temperature profile is used, but we wanted to get a collective temperature range in retort for validating the nonisothermal endpoints method model for predicting vit C retort thermal processing degradation. Any probes that lost connection during processing were not a part of average. Each recipe was altered in cook time and temperature as much as feasibly possible to initiate degradation at extreme times and temperatures and therefore allow interpolation of the degradation parameters from data collected.

The lethal effect ($F_{10/121.1}$) at T = 121.1 °C, z = 10 °C, and F_0 = 1 min was calculated for all temperature profiles. Sugar snap peas $F_{10/121.1}$ values were 6.99, 10.13, and 2.36 for SPLow, SPMod, and SPHigh recipes, respectively. We wanted to target a $F_0 \geq 6$ using the general method to make sure necessary lethality was met to get a minimum 12D kill for *Clostridium botulinum.* In absence of data used to attained F_0, we assumed all values were valid. Since project main initiative was to validate the nonisothermal endpoints method model, we considered this variance insignificant. Rhubarb applesauce $F_{10/121.1}$ values were 0.003, 0.002, and 0.006 for RALow, RAMod, and RAHigh recipes, respectively. Based on (Singh, Singh, & Ramaswamy, 2017), minimum lethality for high acid foods was achieved referencing $F_{10/195}$ = 1.0 mins for pH range 3.3-3.5. The pH was recorded after initial homogenization of pouch sample. Rhubarb applesauce and sugar snap peas pH values were 3.31 and 5.57, respectively.

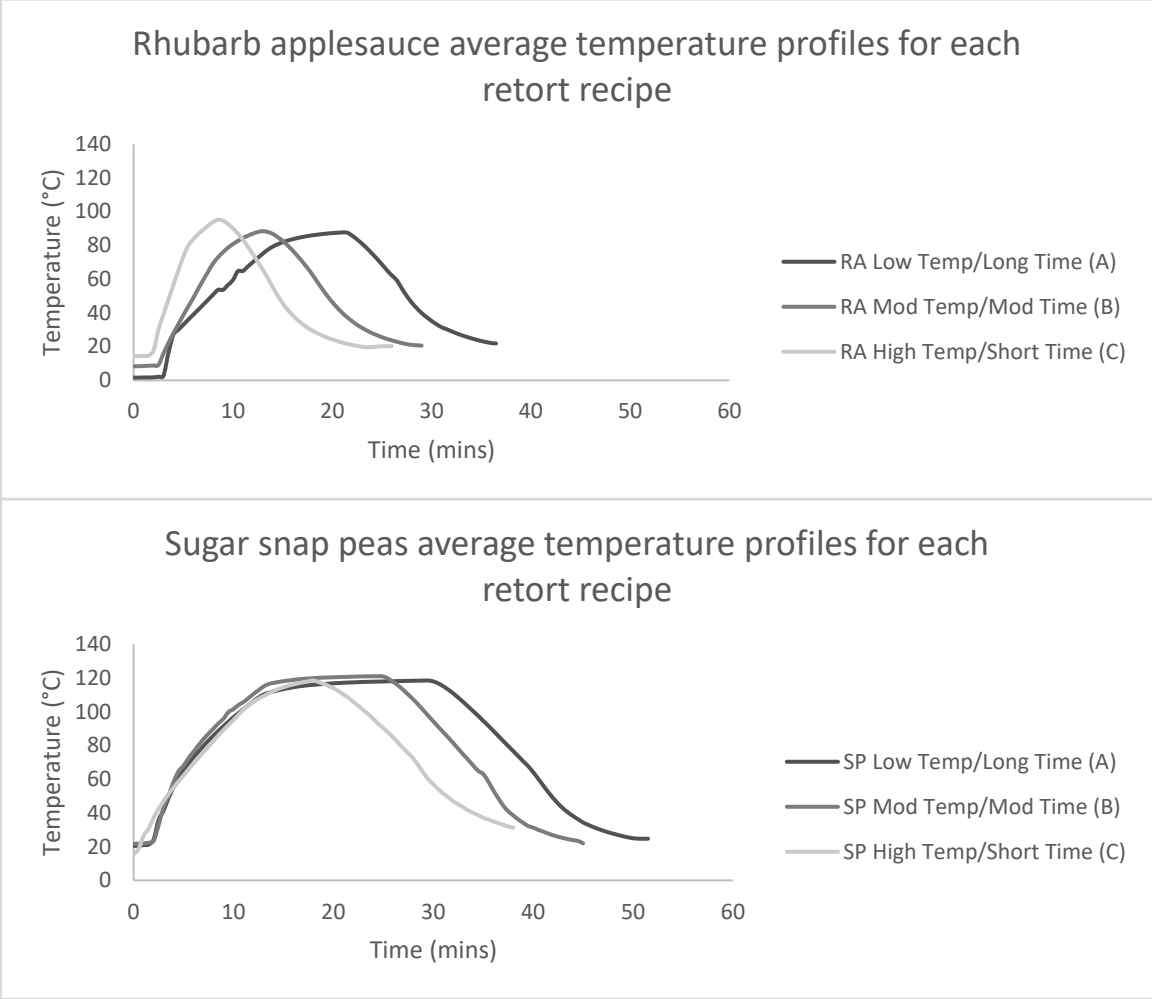


Figure 3.2. Average rhubarb applesauce and sugar snap peas temperature profiles at low temperature/long time (A), moderate temperature/moderate time (B), and high temperature/short time (C).

For rhubarb applesauce, producing extreme retort recipes was more straight-forward due to the lower temperature requirements for acidic foods. However, sugar snap pea retort thermal processing was more limited due to the higher processing temperatures required to obtain our target lethality. Cook time adjustments were therefore more acceptable adjustments to make in producing retort recipes, rather than the temperature. If the experiment were to be repeated, a larger difference in cook temperature across the three

temperature profiles would help differentiate the resulting vit C concentrations, especially for sugar snap peas, which will be further explain vitamin C concentration section. Additionally, repeating each retort recipe would have also been beneficial in determining any error from retort processing, but we considered this error negligible, thus not determining it.

3.4.2. Vitamin C concentration

A detailed description of all vit C concentrations and chromatograms for each food processed across all the temperature profiles are provided in Supp A and B for rhubarb applesauce and sugar snap peas. RALow had the highest decrease in concentration ratio with a 42 % reduction, and SPMod had the highest decrease in concentration ratio at 25 %. Overall, the shorter the processing time, the higher the vitamin retention was for all foods. This trend was most noticeable in rhubarb applesauce. However, sugar snap peas had a similar pattern, but SPLow had the highest retention instead of SPHigh. When the standard deviation was taken into consideration, SPHigh falls within the SPLow range, so it could have statistically been higher if the same experiment was repeated. Based on the FDA's guidelines for determining the number of composite samples necessary for sampling statistical analysis with a 95 % confidence level, six sample replicates were within range.

3.4.3. Vitamin C first-order modeling predictions

The model tested during the study is based on a simplified version of the Arrhenius equation that has proven to be equally effective for discerning degradation parameters during isothermal storage for food vitamins (Peleg, Kim, & Normand, 2015) . To take it a step further, we wanted to unveil its effectiveness with nonisothermal data. Figures 3.3 gives a general outline of the model's effectiveness using non-isothermal temperatures to

predict the vit C concentration with fixed first order kinetics. As discussed before, the letter pairing is just a short-term abbreviation for the temperature profiles used to construct the model's kinetic parameters and discern degradation parameters, which are listed systematically in Table 3.1. The reference temperature was 80 °C for rhubarb applesauce and 100 °C for sugar snap peas. They were chosen from approximating the average temperature of the retort thermal process for all temperature profiles of sugar snap peas and rhubarb applesauce, separately.

As explained earlier, k_{Tref} and c were estimated with two temperature profiles for each possible pair (e.g. AB, AC, and BC) for the three retort recipes Then, the k_{Tref} and c degradation parameters were used to predict the vit C concentration of a temperature profile that was not included in determining the degradation parameters, such as AB kinetic parameters were used to predict C. You can use AC or BC temperature profile to estimate C because the vit C concentration outcome will be the same. When AB predicted C's resulting vit C concentration, the residual from the experimental concentration ratio was 1.4 %. This was one of the best predictions with AC predicting B being the worst predictions at 4.0 % residual for rhubarb applesauce. Overall, the residual average was small at 2.7 %. On the contrary, sugar snap peas had a larger difference between predicted and measured concentrations than rhubarb applesauce with a 7.8% residual average difference. On the contrary, the residual standard deviation was less than 1% for sugar snap peas compared to 1.3% for rhubarb applesauce.

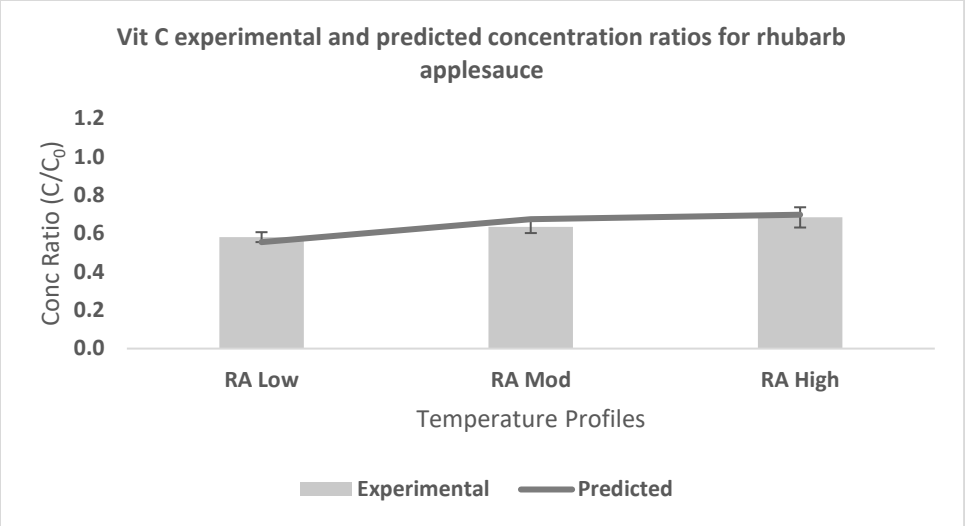
However, there were situations for sugar snap peas where degradation parameters did not produce degradation curves that exactly match the corresponding endpoints. To overcome this, k_{Tref} and c values were selected by matching the degradation curves and

endpoints as well as possible. In the process of achieving this, negative temperature sensitivity degradation parameter, 'c', was used. This was necessary because the experimental data showed that there was a negative temperature relationship with SPLow and SPHigh. The SPLow retort thermal process evidently was exposed to less heat than the SPHigh retort thermal process, but the vit C calculations did not capture this temperature difference. In which, SPLow had a higher vit C concentration than SPHigh, which should not be the case. The results could play a significant role in the higher total error compared to that seen in rhubarb applesauce experiments. Altogether, the predictions were closely aligned with the experimental vit C concentrations, and the predicted points standard deviation were within the experimental CV. All data can be viewed in Table 3.2.

Table 3.2. Using the non-isothermal model to predict vit C concentration with first order kinetics.

Foods	Tref (°C)	Set letter Pair	k_{Tref}	c	Predicting	Observed	Predicted	Residual
Rhubarb Applesauce	80	AB	0.0170	0.0040	C	68%	70%	1.4%
	80	AC	0.0287	0.0440	B	64%	68%	4.0%
	80	BC	0.0183	0.0048	A	58%	56%	2.6%
Sugar Snap Peas	100	AB	0.0011	0.1450	C	79%	87%	8.4%
	100	AC^{+}	0.0035	-0.015	B	75%	83%	7.9%
	100	BC	0.007	0.005	A	79%	72%	7.0%

$^{+}$Pair with degradation curves that was not completely centered on respective concentration dot to determine exact k_{Tref} and c values.
*Set letter pair meanings: A represents a low temperature/short time, B represents a moderate temperature/moderate time, and C represents a high temperature/short time



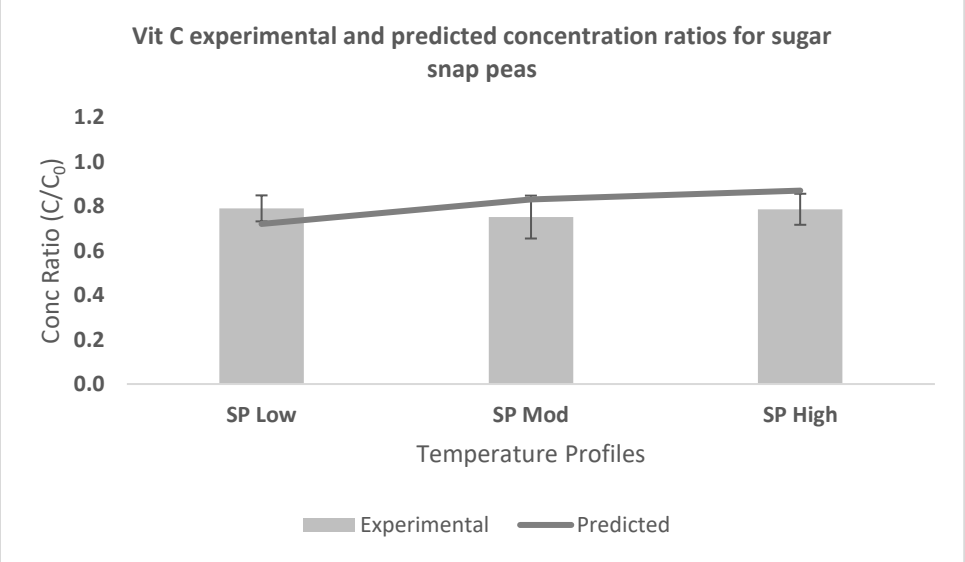


Figure 3.3. Rhubarb applesauce (RA) and sugar snap peas (SP) vit C experimental and predicted concentration ratios for each retort temperature profile.

3.4.4. Manipulating first-order kinetics

Although first order kinetics is the predominant order used in most studies for vit C, we also wanted to get a general idea of what the effects of using zero and second order kinetics would have on the vit C predictions. Unexpectedly, rhubarb applesauce kinetics was better described by second order than first order, evidenced by having an average residual less than 1.0 % compared to 2.7 % for first order kinetics, which can be viewed in

Table 3. With sugar snap peas, first order kinetics had the lowest difference compared to the other suggested orders, but fixed second order kinetics was a close runner-up. Most likely, the food matrix and pH differences played a role in demonstrating different kinetic behavior (Nováková, Solich, & Solichová, 2008). Zero order kinetics was the least accurate for sugar snap peas and rhubarb applesauce. The negative 'c' continued for zero and second order in sugar snap peas, which was expected since the same temperature profiles were being used. However, a new negative 'c' was introduced in AB rhubarb applause, and this developed due to the low lack of degradation curvature and subtle vit C degradation between the temperature profiles.

Table 3.3. Using the non-isothermal model to determine how a ± 1 order can affect vit C concentration predictions.

Foods	Tref	Set letter Pair	n	k_{Tref}	c	Predicting	Observed	Predicted	Residual
Rhubarb Applesauce	80	AB	0	0.0045	-0.025	C	68%	75%	6%
	80	AC	0	0.023	0.06	B	64%	71%	7%
	80	BC	0	0.02	0.02	A	58%	52%	6%
Rhubarb Applesauce	80	AB	1	0.017	0.004	C	68%	70%	1%
	80	AC	1	0.0287	0.044	B	64%	68%	4%
	80	BC	1	0.0183	0.0048	A	58%	56%	3%
Rhubarb Applesauce	80	AB	2	0.021	0.003	C	68%	68%	0%
	80	AC	2	0.035	0.033	B	64%	66%	2%
	80	BC	2	0.0207	0.001	A	58%	58%	0%
Sugar Snap Peas	100	AB	0	0.00096	0.145	C	79%	92%	13%
	100	AC^{+}	0	0.0031	-0.015	B	75%	81%	6%
	100	BC	0	0.0061	0.005	A	79%	71%	8%
Sugar Snap Peas	100	AB	1	0.0011	0.145	C	79%	87%	8%
	100	AC^{+}	1	0.0035	-0.015	B	75%	83%	8%
	100	BC	1	0.007	0.005	A	79%	72%	7%
Sugar Snap Peas	100	AB	2	0.00125	0.145	C	79%	90%	11%
	100	AC^{+}	2	0.0039	-0.015	B	75%	81%	6%
	100	BC	2	0.0078	0.005	A	79%	73%	6%

$^{+}$Pair with degradation curves that was not completely centered on respective concentration dot to determine exact k_{Tref} and c values.

*Set letter pair meanings: A represents a low temperature/short time, B represents a moderate temperature/moderate time, and C represents a high temperature/short time

3.4.5. Adjusting reference temperature

To further examine the model, the reference temperature was also tested ± 15 °C from the original reference temperature used in Table 3.2 and 3.3. Theoretically, changing the reference temperature will change the k_{Tref} term due to the dependent relationship of T_{ref} and k_{Tref}, in which higher T_{ref} values yields higher k_{Tref} values and vice versa. Since there were some negative temperature relationships with sugar snap peas, the correlation was reversed for those points, in which higher T_{ref}, values caused k_{Tref} to decrease. However, the difference had little influence on the residuals, which can be viewed in Table 3.4.

Table 3.4. Using the non-isothermal model to determine how a ± 15 °C in T_{ref} can affect vit C concentration predictions with first order kinetics.

Foods	Set letter Pair	Tref	k_{Tref}	c	Predictor	Actual	Predicted	Residual
Rhubarb Applesauce	AB	65	0.0159	0.004	C	68%	70%	1%
	AC	65	0.0150	0.044	B	64%	67%	4%
	BC	65	0.0171	0.005	A	58%	56%	3%
Rhubarb Applesauce	AB	80	0.0170	0.004	C	68%	70%	1%
	AC	80	0.0287	0.044	B	64%	68%	4%
	BC	80	0.0183	0.005	A	58%	56%	3%
Rhubarb Applesauce	AB	95	0.0178	0.004	C	68%	70%	1%
	AC	95	0.0550	0.044	B	64%	67%	4%
	BC	95	0.0195	0.005	A	58%	56%	2%
Sugar Snap Peas	AB	85	0.0001	0.145	C	79%	91%	12%
	AC	85	0.0044	-0.015	B	75%	81%	6%
	BC	85	0.0065	0.005	A	79%	72%	8%
Sugar Snap Peas	AB	100	0.0011	0.145	C	79%	87%	8%
	AC	100	0.0035	-0.015	B	75%	81%	6%

	BC	100	0.0070	0.005	A	79%	72%	7%
Sugar Snap Peas	AB	115	0.0094	0.145	C	79%	91%	12%
	AC	115	0.0028	-0.015	B	75%	81%	6%
	BC	115	0.0075	0.005	A	79%	72%	8%

[+]Pair with degradation curves that was not completely centered on respective concentration dot to determine exact k_{Tref} and c values.
*Set letter pair meanings: A represents a low temperature/short time, B represents a moderate temperature/moderate time, and C represents a high temperature/short time

3.5 Conclusions

Vit C is a sensitive compound that is highly affected by high temperature exposure, which is straightforwardly observed from the vit C loss following heat processing. The modified endpoints method used to predict vit C concentration post-processing showed promising potential as a reliable platform to help predict vit C loss at different temperature profiles during retort processing and potentially any other form of heat processing where temperature can be tracked and is non-negative. The model could also help reduce experimental calculations needed to reveal degradation behavior. Although first order kinetics is the most common assumption of degradation behavior in literature for tracking vit C degradation kinetics, it can also be beneficial to analyze the possibility of other kinetic orders to ensure that the proposed order is the best representation for modeling degradation in the food being analyzed. In our case, fixed first order kinetics prevailed as the best overall order for predicting vitamin C loss during retort processing.

CHAPTER 4

PREDICTING VITAMIN C LOSS DURING LONG-TERM STORAGE

4.1. Abstract

Vitamin C (Vit C) is a labile compound susceptible to degradation from an assortment of intrinsic and extrinsic factors. To understand the relationships among vit C degradation, time, temperature, and to predict vitamin loss during storage, a model was developed that can derive degradation kinetic parameters, which can describe the degradation behavior of vit C from merely two vitamin concentration endpoints and two constant storage temperatures. In this study, five NASA-utilized space foods were produced, which included rhubarb applesauce (produced three ways with different pH variations), strawberries, and sugar snap peas. Each food was independently retorted and freeze dried to make it shelf stable according to NASA specifications. Samples were stored at three constant temperatures (4, 20, and 37 ºC) and periodically pulled over a two-year period. HPLC analysis was used to measure vitamin C.

Assuming first order kinetics, degradation parameters were determined and then degradation curves were constructed using average and individual degradation parameters from each temperature combination and 4 and 20 ºC as a single pair. Overall, first order kinetics alone was not good enough to consistently get reliable predictions. Thus, a combined first order kinetics model was used, and a database was made from experimental data to allow users to estimate degradation parameters with unknown foods by inputting a few general physiochemical properties (pH, moisture content, and storage temperature). Likewise, 4 and 20 ºC final endpoints database was also created. Both

databases had potential to be a resourceful tool for finding degradation parameters without doing a storage study.

4.2. Introduction

Travelling in space is a taxing task that needs extensive planning to ensure astronauts' safety. Proper meal planning to promote a healthy balance diet is a vital part to begin this process. Fruits and vegetables are key components to meet human nutritional requirements, especially micronutrients. During long-term spaceflight, micronutrients, such as vitamins, are constantly degrading due to an array of variables, including temperature, light, oxygen, pH, time, food matrix, and many more intrinsic and extrinsic factors. These factors are most susceptible to water soluble vitamins, and vitamin C (vit C) has shown many degradation vulnerabilities to all the listed variables (Burdurlu, Koca, & Karadeniz, 2006; Catauro & Perchonok, 2012). On earth, there are reliable sources for replenishing food; however, during long-term space trips, such as a trip to Mars, this refueling task is impractical making it crucial to understand how the vitamins are degrading to develop a diet that meets astronauts' nutritional requirements at any time point in space. To help determine this, our team took a closer look at how rhubarb applesauce (RA), strawberries (ST), and sugar snap peas (SP) degradation kinetic behave under different processing vessels and storage conditions.

RA, ST, and SP are a few of the many NASA shelf stable recipes. These recipes were chosen to represent the main food groups that has vit C, which predominantly includes fruits and vegetables with fruits typically having more vit C (Kaur & Kapoor, 2001). Post food preparation, foods were processed by retort processing or freeze drying. The acidity played a huge role in determining processing conditions for the retort. Where

the high acid foods, rhubarb applesauce and strawberries, were processed at a lower temperature and time compared to the low acid foods, which has a pH $\geq$ 4.6 (Gavin & Weddig, 1995). Moreover, this study will allow readers to juxtapose retort and freeze dry kinetics in three different food matrices along with a determining a reliable method to make vit C predictions during long-term storage at three constant temperatures.

Gathering vit C degradation kinetics was done with a model built in-house using Wolfram Mathematica. Users can freely access the program on http://demonstrations.wolfram.com/. The user only needs to type "endpoints method" in the search engine, and the first two results will show the "Prediction of Isothermal Degradation by the Endpoints Method" model and the "Endpoint Method for Predicting Chemical Degradation in Frozen Foods" model ("Wolfram Demonstrations Project," n.d.). This project used the first model because only positive temperatures were used for predictions. The models have the capability of predicting the concentration ratio of any temperature at any time with only knowing the initial concentration of the studied compound, which is vit C in our case; however, the frozen model can only predict negative temperatures and vice versa for the other model. The theory/calculation section will provide an in-depth overview of the model. The model is based on a simplified version of the Arrhenius equation. It has been vetted with published data sets showing how effective it can be (Peleg, Normand, & Kim, 2014). A combined first order kinetics model was also created to enhance the endpoints method model to encapsulate experimental data (Phillips, Council-Troche, McGinty, Rasor, & Tarrago-Trani, 2016; Righetto & Netto, 2006). Our results will help further understand the relationship of physiochemical food properties and vitamin C degradation kinetic parameters to assist

astronaut scientists in making informative decisions on determining vitamin C content in foods at any point during long-term spaceflight.

4.3. Materials and methods

4.3.1. Analytical material

Vitamin C (Vit C) standards were referenced from L-Ascorbic Acid (99% purity) purchased from Fisher Scientific. Vit C extraction from food utilized three extraction stabilizers: TCEP hydrochloride (reducing agent) purchased from Thermo Scientific, ethylenediaminetetraacetic acid (EDTA) disodium salt dehydrate (chelator) purchased from Sigma-Aldrich, metaphosphoric acid (MPA; pH reducer; 33.3-36.5% HPO3 purity) purchased from Reagent World, Inc., and single-distilled laboratory water. The mobile phase (MP) for HPLC analysis consisted of 4 reagents: EDTA (99% purity) purchased from Acros Organics, sodium acetate (pH 3.0+0.1) purchased from J.T. Baker Chemical Co., dodecyltrimethylammonium bromide (DTAB; 99% purity), phosphoric acid (pH adjuster; 85-90% purity) purchased from Fluka Analytical, acetonitrile (column cleaning/storage solvent) purchased from Fisher, and triple deionized laboratory water.

4.3.2. Food material

Stringless sugar snap peas (Mann Packing Co., Inc.), frozen rhubarb, unsweetened canned applesauce (West Creek), and sliced strawberries 4+1 (Simplot Classic) were purchased from Performance Food Service (One Performance Boulevard, P.O. Box 3024, Springfield, MA, USA). Sugar snap peas gravy ingredients: butter, noniodized salt, cornstarch, and ground black pepper were all purchased from local grocery store. Tap water was used to make gravy. Baking soda and citric acid were also purchased from local grocery store and used to adjust rhubarb applesauce pH.

4.3.3. Packaging material

Retort pouches were opaque aluminized pouches with a thermal seal coating [12.065 cm x 20.48 cm (4.75” x 8.0625”); Tan PE/.0007Foil/3mil Coex Sealant] that were purchased from Heritage Packaging (441 Market St, Lawrence, MA 01843, USA). Freeze dry packaging material consisted of a clear primary MB 225L pouch [12.7 cm x 22.225 cm (5” x 8.75”); 225 microns nylon/EVOH/enhanced linear low density polyethylene] purchased from Winpak (100 Saulteaux Crescent, Winnipeg, MB R3J 3T3, Canada), and a white opaque secondary pouch [17.78 cm x 30.48 cm (7” x 12” OD); Seals: 0.9525 cm (3/8”); 1/48ga PET/98ga White OPP/.00035 Foil/2mil LLDPE with tear notches at 1.905 cm (3/4”) purchased from Technipaq (975 Lutter Dr, Crystal Lake, IL 60014, USA). VacMaster SVP 20 (Overland Park, KS 66211, USA) at 1.016 bar (~30 in. Hg) was used to seal pouches. Additionally, Glad sandwich zipper bags [The Glad Products Co., 1221 Broadway, Oakland, CA 94612; 6-5/8” x 5-7/8” (16.8 x 14.9) cm] were purchased from local grocery store and were used to process freeze dry samples.

4.3.4. Rhubarb applesauce, strawberries, and sugar snap peas preparation

Rhubarb Applesauce contained three ingredients: unsweetened applesauce (40%; w/w), frozen diced rhubarb (40%; w/w), and frozen strawberries 4+1 (20%; w/w). Applesauce was manually mixed with blended strawberries and rhubarb was folded into mixture until uniform. Final product content was filled in retort pouches with a minimum fill weight of 142 g and a maximum fill weight of 156 g. Strawberries contained two ingredients: frozen strawberries 4+1 (99.93%; w/w) and ascorbic acid (0.07; w/w). Ingredients were thoroughly mixed until the ascorbic acid was uniformly dispersed in the strawberries. Prior to mixing ingredients, frozen strawberries were thawed for 24 hours in

refrigerator. To get desired ratio, 0.32 g of ascorbic acid was added for every pound (~454 g) of strawberries. Final product content was filled between 148 and 152 g in a retort pouch. Sugar snap peas 6.4 kg (~14 lbs) were blanched in boiling water at 100 °C (212 °F) for 3 minutes in kettle and immediately submerged in ice-cold water at 0 °C (32 °F) until cool. Next, a starch slurry gravy was made to coat the sugar snap peas. Butter 0.22 kg (~0.5 lbs.) and 1,048 mL of water were melted and mixed in a large stainless-steel pot on medium high until content temperature was above 81.7 °C (170 °F). Salt (55 g), cornstarch (69 g), and black grounded pepper (7 g) were mixed in a separate bowl. After mixing, 135 mL ambient temperature tap water was added to mixture slowly. This mixture was mixed into the water/butter solution on the stove recorded over 81.7 °C (170 °F). Once added, the product was heated to 81.7 °C (170 °F) again and was held at 170 °F for 3-5 minutes. When the starch slurry gravy was completed, a Bostwick consistometer was used to verify a consistency of 16.5 cm $\pm$ 0.5 cm in 15 seconds at 81.7 °C (170 °F). Once the consistency parameters were met, the gravy was coated on the sugar snap peas. According to NASA specifications, sugar snap peas were filled into each retort pouch at a minimum weight of 100 g and a maximum weight of 114 g. Between 14 and 21 g of the starch slurry gravy was added to each retort pouch. Pouches were labelled, and final weights were recorded. All pouches from each food were vacuum sealed with VacMaster SVP 20.

4.3.5. Experimental Design

To conduct a two-year experimental storage study with five food products: rhubarb applesauce, (RA), RApH3, and RApH4, strawberries (ST), and sugar snap peas (SP), three storage temperatures (4, 20, and 37), multiple pull dates (3 mo intervals for 20 & 37 °C and 4 mo intervals for 4 °C), two processing methods (retort and freeze drying),

and six sample replicates; our team process over 1,300 pouches for each processing method plus additional pouches to measure initial vitamin C (vit C) concentration prior to processing and for probing to track temperature during each retort run. A general overview of experimental designed is delineated in Figure 4.1.

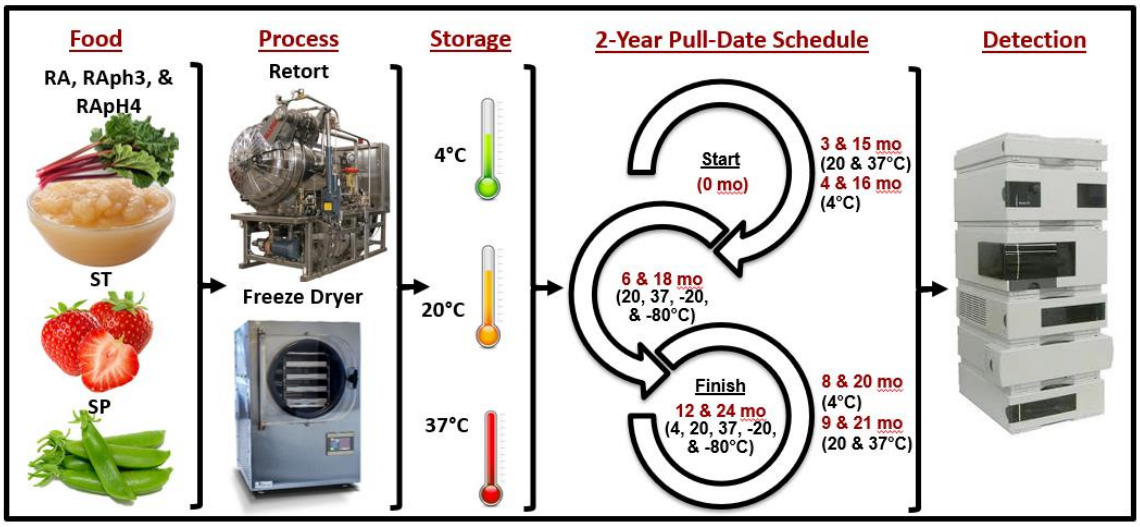


Figure 4.1. Two year experimental storage study design. RA represents rhubarb appleasauce; ST represents strawberries; and SP represents sugar snap peas.

4.3.5.1. Retort Thermoprocessing

Two retort processes or an industry speak, "recipes," were used to process all five foods, which were separated based on pH levels. The retort recipe was an in-house recipe that followed NASA specifications, which required a minimum cook at 91.3 °C (200 °F) for 2 mins for all variations of rhubarb applesauce and strawberries (high acids foods) and $F_0 = 6$ or equivalent lethality for sugar snap peas (low acid food). Four batches were processed with the retort for each food due to its 42-pouch capacity. Each sample was retorted using an Allpax 2402-R3 R&D horizontal batch retort (Allpax Products, LLC, 13510 Seymour Meyers Blvd., Covington, LA 70433 USA), using the water spray method. Additionally, an HH378 Omega data logger (OMEGA Engineering, INC., 800

Connecticut Ave., Suite 5N01, Norwalk, CT 06854 USA) was used to track sample temperature data in 30 s intervals, and Se379 software (Cetani Corporation, 11495 N Pennsylvania St Suite 240, Carmel, IN 46032 USA) recorded the time-temperature data on the computer in comma separated value format. Each run had four interspersed probes within the retort vessel to provide the non-isothermal temperature profiles to verify minimum cook specifications.

4.3.5.2. Freeze Drying

Likewise, two different freeze dry recipes were used for the foods that followed the same categorical separation aforementioned in the retort section. The high acid foods freeze dry recipe was an in-house recipe modified from NASA's strawberry freeze dry recipe, and the low acid food recipe was also an in-house recipe modified from NASA's spicy green beans recipe. Prior to processing, retort pouches were removed from -80 °C and samples were shattered to increase surface area. The sample pieces were placed in a Glad zipper bag and frozen at -40 °C for at least one hour. Then, three batches were processed with the freeze dryer due its 48-sample capacity and no probing were utilized. Each sample was freeze dried using an Genesis Pilot Lyophilizer (SP Scientific, 3538 Main St., Stone Ridge, NY 12484).

Post processing and packaging for freeze drying and retort thermoprocessing, all pouches were stored in their respective temperature (4, 20, and 37 °C), and six samples were stored at -80 °C for each process to measuring the initial vit C concentration. Over a two-year span, six replicates at 20 and 37 °C were pulled every 3 mo and 6 replicates at 4 °C was pulled every 4 mo. Since all samples were measured in quadruplicates, the two remaining samples were stored in -80 °C for back up.

4.3.6. Retort thermoprocessed food sample analysis preparation

Preparation began with blending the entire retort pouch content in a 250 mL stainless steel blender cup using a Waring blender on low intensity for 15 s to ensure a smooth, homogenous blend during this process. Post-blending, 2 mL bead ruptor tubes (tubes designed for use with bead ruptor homogenizer) were filled with 4 (2.8 mm) ceramic beads (beads were specifically designed for bead ruptor to pulverize and separate content). Then, an aliquot of 0.5 g was added to tube for rhubarb applesauce, and 0.3 g for sugar snap peas and strawberries. Extraction reagents (EDTA disodium salt (5%), TCEP (0.1 %), 6% MPA (5%), and triple deionized water (89.9%) were added in a 1 mL proportion for rhubarb applesauce and 1.5 mL proportion for strawberries and sugar snap peas to obtain proper dilution based on expected vit C content. Samples were blended with Omni bead ruptor 24 (Omni International, Inc., 935-C Cobb Place Blvd. NW Kennesaw, GA 30144 USA) homogenizer for 2 mins on max intensity. The specific program was set to S = 8.00 (max speed), T= 10 s (shake time), C = 3 (# of cycles), and D = 30 s (rest time). This cycle was used to maximize extraction efficiency while minimizing heat production. The samples were then centrifuged at 14,000 RPM for 15 mins at 4 °C to separate fibrous content. Prior to HPLC analysis, samples were filtered through a 13 mm, 0.45 μm nylon filter membrane. Strawberries were further diluted with extraction buffer (1:10). Standards of 2, 7, and 30 mg/L were used to construct a standard curve and convert reported intensity areas from HPLC to known vit C concentrations.

4.3.7. Freeze dry food sample analysis preparation

Freeze dry preparation began in a similar fashion as retort thermoprocessed food preparation. However, the entire retort pouch sample was diluted with single-distilled

water prior to blending on low intensity for 15 s where the sample was diluted to its original wet state. This value was calculated from experimentally determining water loss during freeze drying and by recording moisture content values of each food post freeze drying. Additionally, the sample amount in bead ruptor tubes were all reduced to 0.3 g for each food. The remaining steps were the same as the retort thermoprocessing steps aforementioned above.

4.3.8. Vitamin C determination

Vitamin C (Vit C) content was determined experimentally by using a modified version of the AOAC Official Method 2012.21, "Vitamin C in Infant Formula and Adult/Pediatric Nutritional Formula with UV Detection" (Schimpf, Thompson, & Baugh, 2013) where Vit C was detected using an HPLC Agilent Technologies 1100 series with a VWD detector (g1314A), degasser, and binary pump. Agilent OpenLab CDS ChemStation Edition was used to record data. The detector was set to 254 nm. A Synergi Polar-RP, 2.5 μm, 100 Å, 3 × 100 mm column from Phenomenex was used for separation. The flow rate was set to 0.4 mL/min, injection volume (20 μL), and a 15 min run time was sufficient for analysis. Post analysis, HPLC lines and column was rinsed with water/ACN (95/5 %) to removed salts and an increasing gradient to 100% ACN to store column and remove any impurities.

4.3.9. Vitamin C degradation kinetics model theory

Once the vit C concentration ratio (C_t/C_0) at two temperature endpoints is known, the "Prediction of Isothermal Degradation by the Endpoints Method" was utilized to predict the concentration ratio at another time-temperature point. The mathematics behind the model originates from rate law equation:

$$\frac{\mathrm{d}C(t)}{\mathrm{d}t} = -k[T(t)]C(t)^n \qquad (1)$$

where k[T(t)] is the rate constant referenced at a set temperature and time, C(t) is the momentary concentration of vit C at time (t), and n is the reaction order. Vit C storage project focused on first-order kinetics due to many studies successfully using this order to model vit C (Cruz, Vieira, & Silva, 2008; Giannakourou & Taoukis, 2003; Polydera, Stoforos, & Taoukis, 2003). When n = 1, the isothermal solution from equation 1 can be written as

$$\frac{C_{(t)}}{C_0} = e^{[-\mathrm{k(T)t}]}. \qquad (2)$$

From the Arrhenius equation, a temperature dependent rate exponential equation was developed:

$$k(T) = k\left(T_{ref}\right)e^{[c\left(T-T_{ref}\right)]}, \qquad (3)$$

where t represents time (s), k_{Tref} is the rate constant at a set reference temperature (T_{ref}), T is the isothermal temperature, and c is a temperature sensitivity constant (Peleg & Normand, 2015). c is related to activation energy (E_a) in the Arrhenius equation that can be converted to E_a with the following equation:

$$E_a \approx cR(T_{ref} + 273.16)^2 \qquad (4).$$

With equation 3 inserted into equation 2, the two experimental concentration ratios (C_1 and C_2) at t_1 and t_2 using constant temperatures at T_1 and T_2, above the freezing mark, can algebraically determine the concentration ratio at any isothermal endpoint. The output will yield equation 5 and 6.

$$C_1 = e^{-k_{Tref}{}^{e^{c(T_1-T_{ref})}}t_1} \qquad (5)$$

$$C_2 = e^{-k_{Tref}{}^{e^{c(T_2-T_{ref})}}t_2} \qquad (6)$$

Equation 5 and 6 can also be numerically solved to determine k_{Tref} and c to predict the concentration at other time-temperature points and ultimately reconstruct an entire degradation profile. This exponential model has been theoretically and experimentally tested to be a reliable tool for predicting labile vitamins storage concentrations (Peleg, Normand, & Corradini, 2017).

To enhance the first order kinetics endpoints method model's efficacy, combined first order kinetics model was also utilized post two-year storage study. The combined first order kinetics model is based off the principle that vit C or more specifically total vit C follows two degradation pathways: aerobic and anaerobic degradation. Creating two temperature-dependent rate constants, $k_{\text{aerobic}}(T)$ and $k_{\text{anaerobic}}(T)$ where

$$C(t) = C_{\text{aerobic}} e^{-k_{\text{aerobic (T)}} t} + (1 - C_{\text{aerobic}}) e^{-k_{\text{anaerobic(T)}}\, t} \qquad (7)$$

$C(t)$ is the momentary fraction of vit C and C_{aerobic} is the fraction of the original vit C concentration, which is degraded by the aerobic mechanism where vit C bypass dehydroascorbic acid (DHAA) during degradation and tends to follow first order kinetics). The anaerobic mechanism is where vit C degrades to DHAA first and has a slow degradation due to DHAA being biologically active vit C. Equation (7) can be further simplified to Eq. 8 when the two mechanisms occur simultaneously.

$$C(t) = C_{asymp} + (1 - C_{asymp}) e^{-k_{aerobic}(T)t} \qquad (8).$$

C_{asymp} is the asymptotic concentration fraction of the original vit C. Overall, the combined first order kinetics equation degradation curve starts with exponential decay and ultimately decay to a nonzero residual value for a discrete period of time. The variable, C_{asymp}, played a vital role in improving vit C's fit for many of the tested foods.

4.3.10. Statistical Analysis

All vit C concentrations were expressed as mean ± standard deviation (SD) across the four replicates for each food. The coefficient of variation and a 0.95% confidence interval was used to compute sample size and minimize relative error. RStudio was utilized to perform nonlinear regression analysis with experimental data to determine significance of degradation parameters (C_{asymp}, k(T), k_{Tref}, and c).

4.4. Results and discussion

4.4.1. Food vitamin C concentrations

Sugar snap peas (SP), strawberries (ST), rhubarb applesauce (RA), rhubarb applesauce pH3 (RApH3), and rhubarb applesauce pH4 (RApH4) were analyzed to determine vit C concentrations, utilizing retort thermoprocessing (TP) and freeze drying (FD), under storage at 4, 20, and 37 °C covering a 24 month span with eight pull dates for 20 and 37 °C that was pulled every three months and six pull dates for 4 °C that was pulled every four months. The raw concentration results for each process and storage temperature can be visually viewed in Supp C. in appendix. To help abridged the writing, the temperature, process, and food was abbreviated throughout this section in the aforementioned order. For example, "37RAFD" refers to rhubarb applesauce at 37 °C processed by freeze drying. If one section of the nomenclature is not included, then assumed all variables are being referenced, such as "RAFD" refers to all RAFD temperatures.

As expected, FD preserved the vitamin better throughout all foods under all temperatures except 37 ºC (Uddin, Hawlader, Ding, & Mujumdar, 2002). The degradation rate was the most noticeable difference because in many cases FD caused the food to approach a zero residual value quicker than TP foods. However, the FD values

started with a higher vit C amount. As long as the vit C was detectable/nonzero, FD still had more vit C content in all the foods. This finding stress how the initial concentration and an elevated storage temperature can influence vit C degradation. Sugar snap peas had many bewildering findings for TP and FD. Specifically for TP, the vitamin concentration considerably declined for all temperatures and was undetectable for all temperatures before the end of the two-year storage study. The stability was better during FD, but a sharp drop occurred at 12 mo for 20SPFD, which seemed valid considering the remaining time points stabilize near that concentration. The cause of the dropped has yet to be determined, and we assume there had to be some form of phenomenon that increased vit C dissolved oxygen leading to a more rapid degradation (Gómez Ruiz, Roux, Courtois, & Bonazzi, 2018).

All the acidic foods, RA and ST had higher stability during TP and FD, especially FD where 4 and 20 ºC had negligible degradation during the two-year storage study. However, RApH4 experienced a more rapid degradation rate due to the increased pH level. There was a similar trend with RApH3, except reduced degradation rate, due to the lower pH level.

4.4.2. Model Data Processing

With vitamin C (vit C) concentrations determined for all foods, the endpoints method model was used to predict vitamin concentration ratios. To fully grasp model functions, one example from project data will be demonstrated. First, vit C concentrations determined from storage study should be converted to concentration ratios. Then, insert any two experimental temperatures, time, and concentration ratio profiles in the kinetic parameter estimation section. For the example in Figure 4.2, 4RA at 120 days (4 mo) and

20RA at 90 days (3 mo) were chosen. Once the parameter estimation section is populated, an isothermal temperature graph will display on top, and the concentration ratios values will populate at the bottom of the interface on the right-hand side. Based on prior data, first-order kinetics was chosen, but this can be manipulated to best suit degradation. Additionally, post two-year storage study, it was confirmed that first order kinetics provided the lowest average coefficient of determination value among zero and second order fixed kinetics. Reference temperature was set to 25 °C due to its proximity to all the storage temperatures. User friendly sliders were used to determine k_{Tref} and c values by matching corresponding concentration ratios with color matching degradation curves, which is shown in Figure 4.2. If the time exceeded 320 days, then the user can use the t_{max} slider to adjust the time scale. Time units are arbitrary, so unit adjustments can be changed and still yield the same results but must continue to use same units when making predictions. The last input panel allows the user to make predictions. When desired time-temperature values are inputted and checkbox is checked, the concentration ratio will be displayed above temperature profile graph, and an entire degradation curve is produced on the reconstructed degradation curves graph showing all interpolated and extrapolated concentration ratios, which is discerned in red in Figure 4.2 for all prediction content.

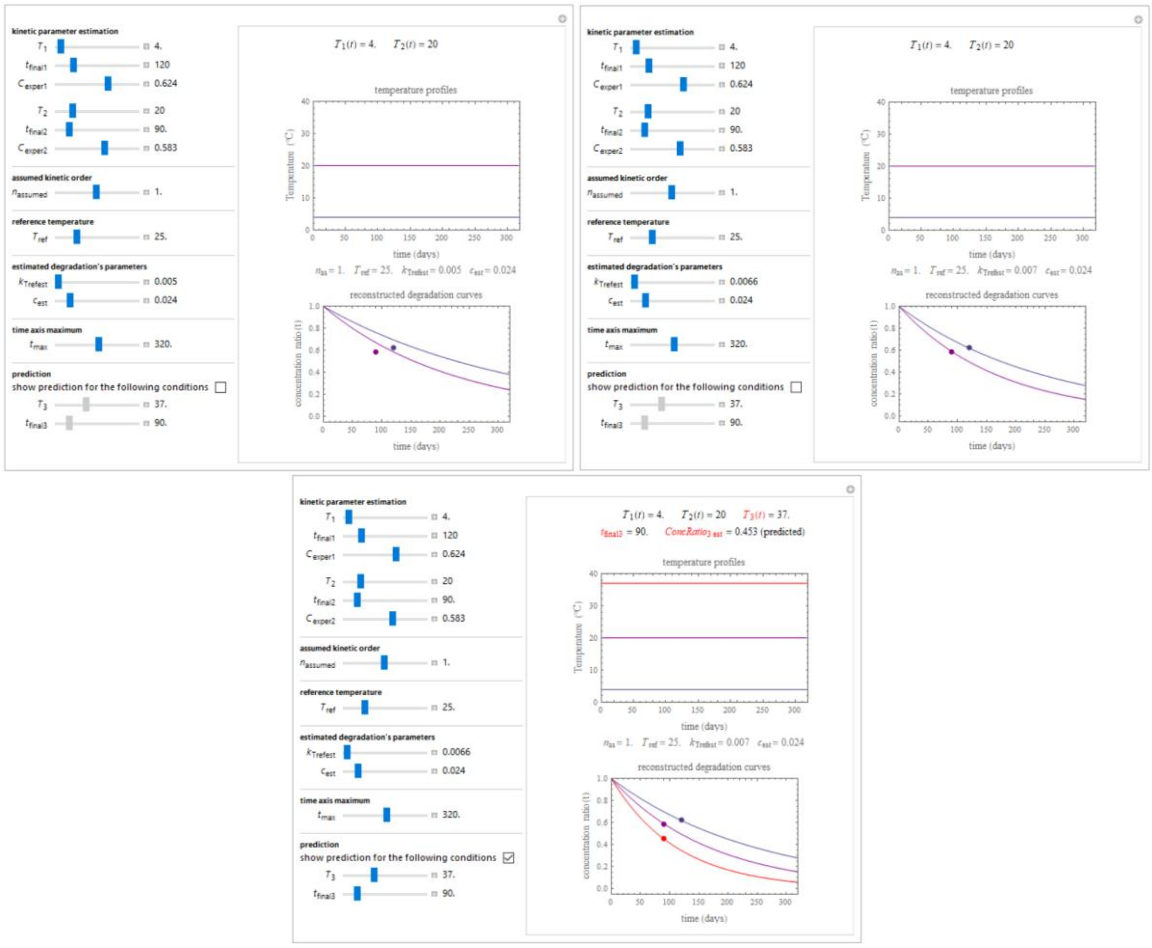


Figure 4.2. Left: unmatched k_{Tref} and c degradation parameters for rhubarb applesauce using 4 and 20 ºC storage data at 4 and 3 months, respectively; right: matched k_{Tref} and c degradation parameters for rhubarb applesauce using 4 and 20 ºC storage data at 4 and 3 months, respectively; and bottom: using matched k_{Tref} and c degradation parameters for rhubarb applesauce to predict vit C concentration at 37 ºC for 3 months.

However, this model focused only on predictions. Post two-year storage study, data was optimized by utilizing the combined first order kinetics model to determine C_{asymp} and the temperature dependent rate constant, k(T), for each storage temperature. The rate constant at each temperature (4, 20, and 37 ºC) was used to determine the optimal k_{Tref} and c constants to make predictions. The effectiveness of model will be further explained in the combined first order kinetics predictions section. Additionally, final experimental endpoints at 4 and 20 ºC were used to summarized data as well.

4.4.3. Vitamin C degradation curves

The goal of the endpoints method model is to be able to use minimum experimental data to predict long-term vit C concentration in a variety of food systems with limited margin of error ($\leq$ 15% residual average and standard deviation). In order to achieve this, early timepoint kinetic parameters from experimental data were used to construct degradation curves. Figures 4.3-4.9 show example degradation curves using 4 different time points (3,4 mo; 6,8 mo; 9,12 mo; and 12, 12 mo) along with one degradation curve using combined first order kinetics encompassing all data points represented as C_{asymp}.

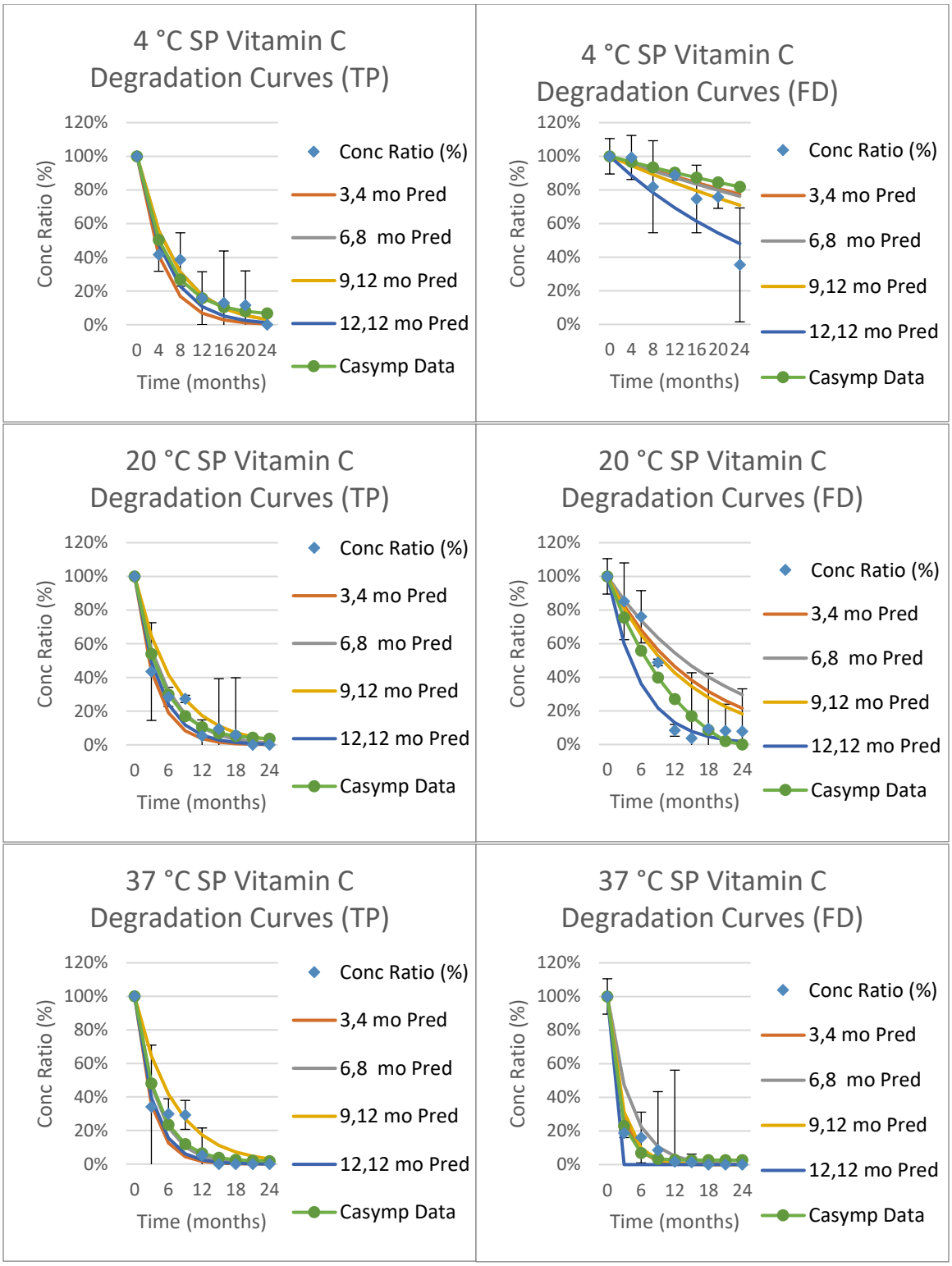


Figure 4.3. Sugar snap peas (SP) retort thermoprocessed (TP) and freeze dried (FD) predictive degradation curves, experimental concentration ratio percent values, and combined first order kinetics fit over a 24 month time frame at 20, 37, and 4 ºC storage temperatures.

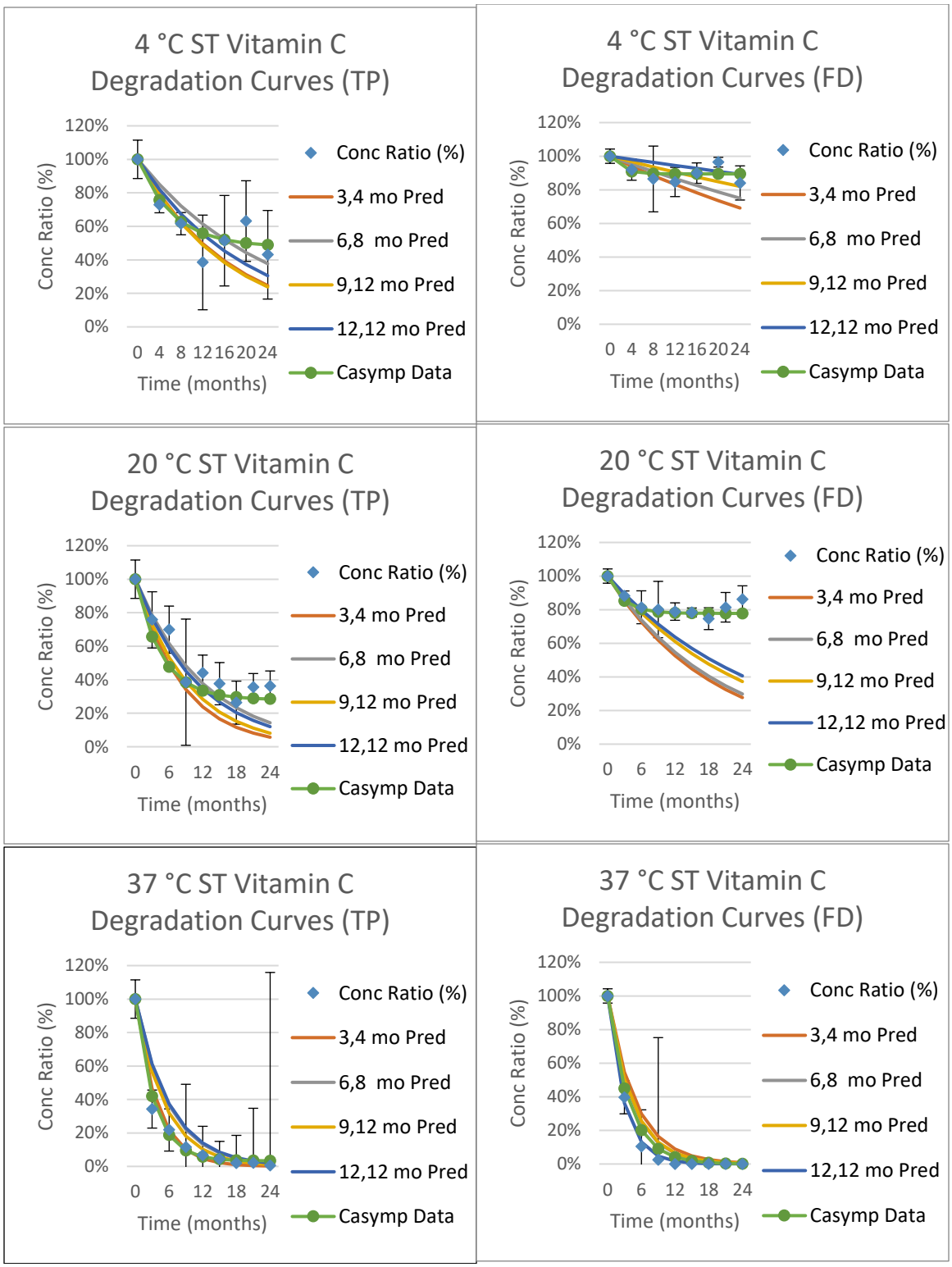


Figure 4.4. Strawberries (ST) retort thermoprocessed (TP) and freeze dried (FD) predictive degradation curves, experimental concentration ratio percent values, and combined first order kinetics fit over a 24 month time frame at 20, 37, and 4 ºC storage temperatures.

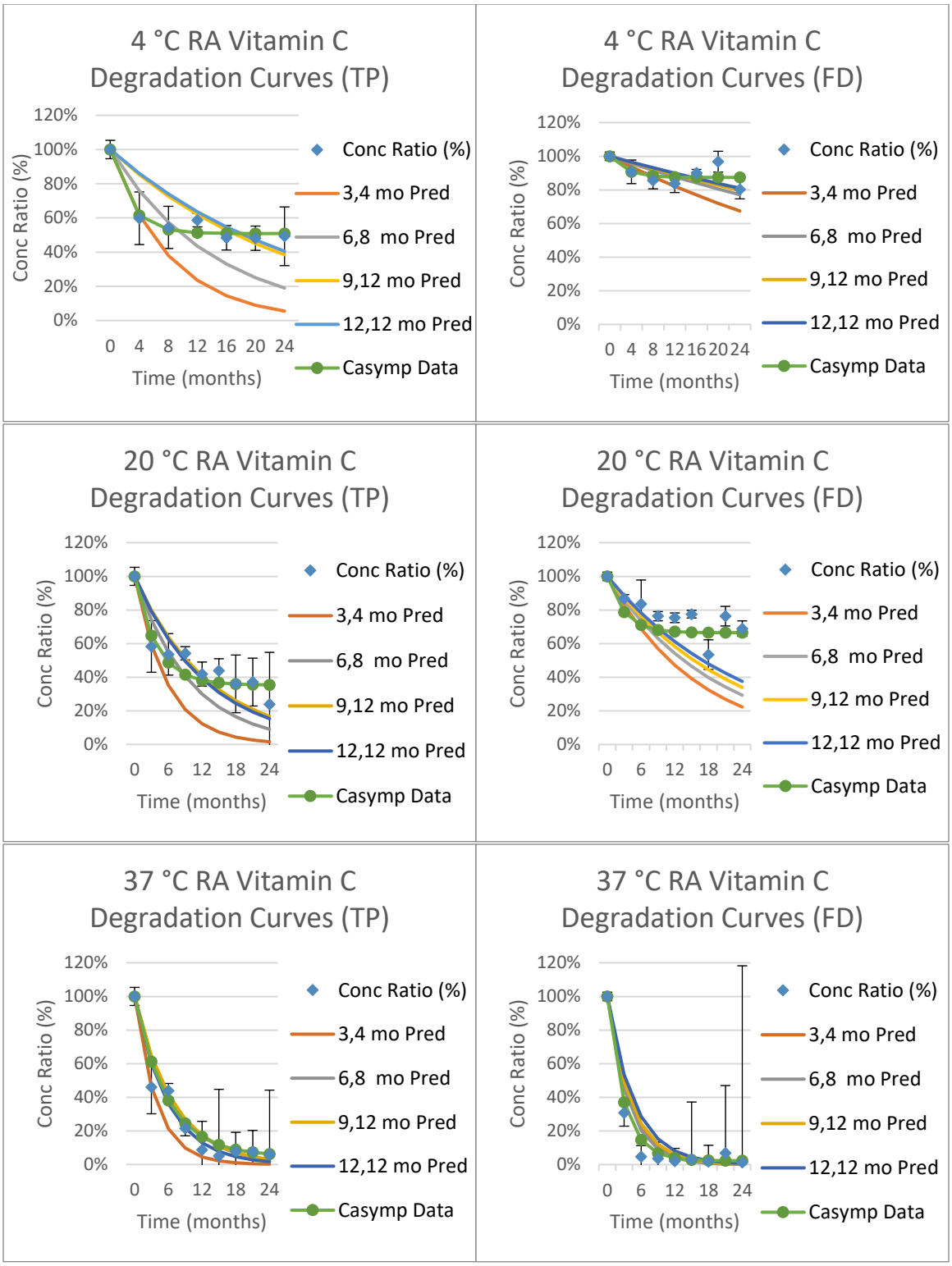


Figure 4.5. Rhubarb Applesauce (RA) retort thermoprocessed (TP) and freeze dried (FD) predictive degradation curves, experimental concentration ratio percent values, and combined first order kinetics fit over a 24 month period at 20, 37, and 4 ºC storage temperatures.

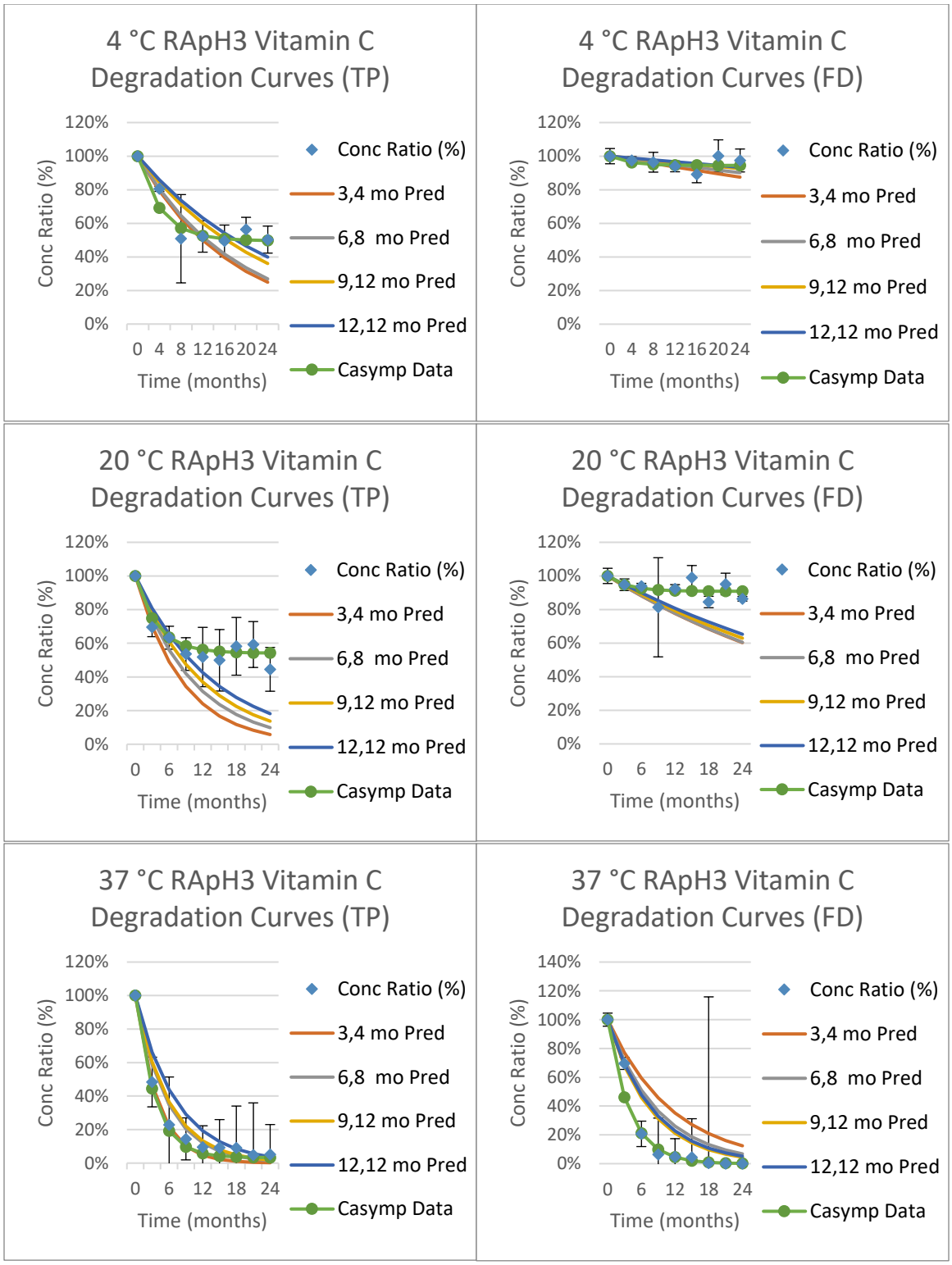


Figure 4.6. Rhubarb Applesauce (RA) pH3 retort thermoprocessed (TP) and freeze dried (FD) predictive degradation curves, experimental concentration ratio percent values, and combined first order kinetics fit over a 24 month period at 20, 37, and 4 ºC storage temperatures.

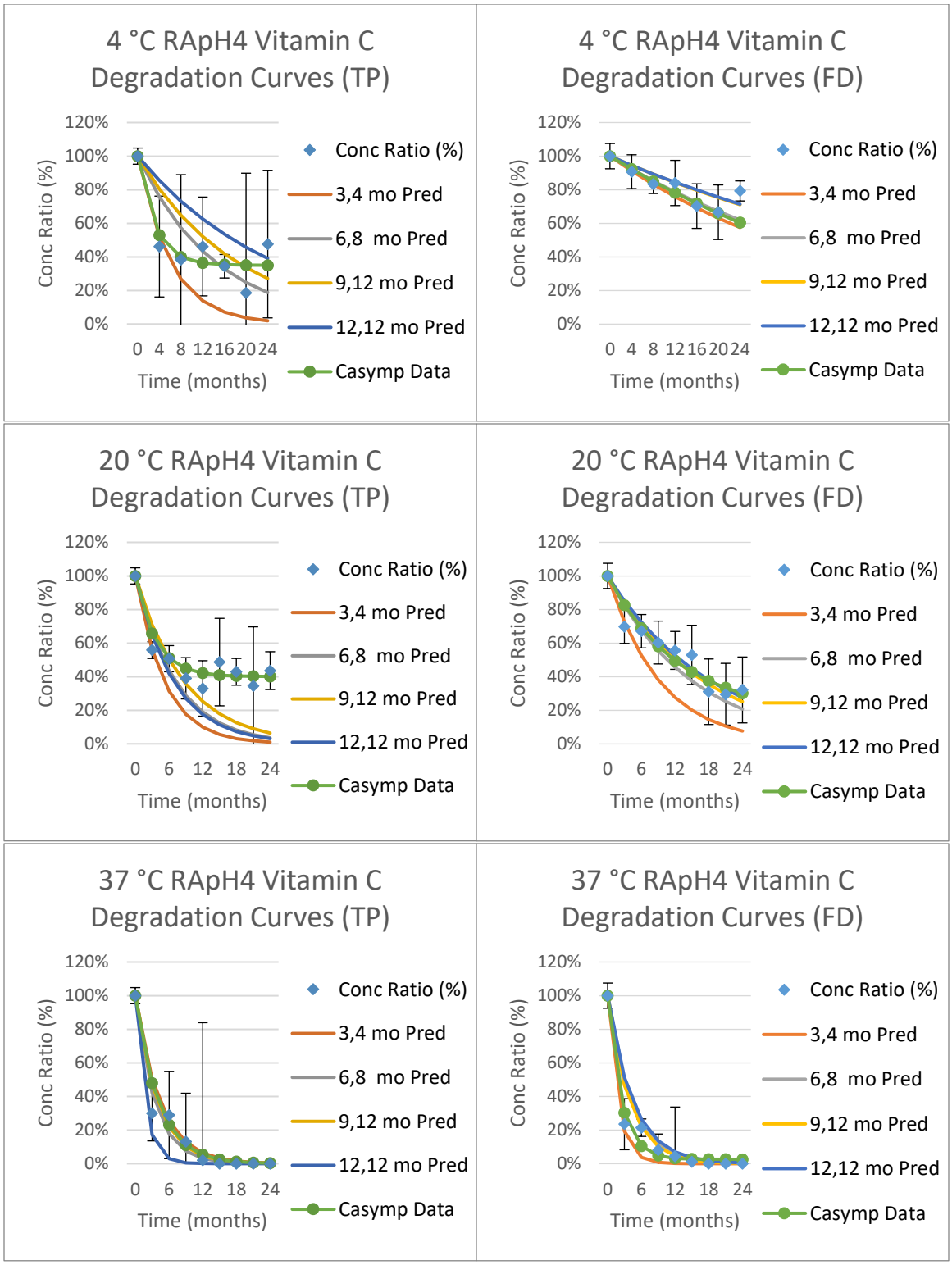


Figure 4.7. Rhubarb Applesauce (RA) pH4 retort thermoprocessed (TP) and freeze dried (FD) predictive degradation curves, experimental concentration ratio percent values, and combined first order kinetics fit over a 24 month period at 20, 37, and 4 ºC storage temperatures.

The kinetic parameters utilized to construct the degradation curves are not lone values from one time-temperature data pair, which is the minimum amount of data points needed for model usability. Each time point is based on 3 pairs of time-temperature data points, and the average k_{Tref} and c from each pair was used to construct the degradation curve. For example, the 3,4 mo pair would consist of degradation kinetics from 20 °C at 3 mo and 4 °C at 4 mo for one pair, 20 °C at 3 mo and 37 °C at 3 mo for another pair, and 4 °C at 4 mo and 37 °C at 3 mo for the last pair. This trend was repeated for 6,8; 9,12; and 12,12 mo, except the months were changed to the respective months in scheme, such as 6,8 predictions would use 6 and 8 mo data and so on for the other predictions. With more pairs, outlier can potentially be removed, but three pairs were not substantial enough to statistically remove any outliers; however, pairs with 37 °C did tend to overpredict the degradation. The data summary will primarily focus on 20 °C predictive degradation curves since this is the closest shelf stable temperature, which is the predominate storage temperature for food during spaceflight.

Referencing Figure 4.3, the predictive degradation curves for 20SPTP provided less than 7% residuals average (RAvg) for all predictive points complemented with a less than 8% residual standard deviation (RSD). Most of the time, it underpredicted the degradation. It is better to overpredict to allow users to over compensate on vit C requirements than under compensate; additionally, vit C has very little toxicity concerns in higher concentrations (Frei, Birlouez-Aragon, & Lykkesfeldt, 2012), but achieving predictions near the experimental data is even more important. However, the results had a trend to under predict and were much less accurate for 20SPFD. The RAvg and RSD fluctuated between 10 and 20%. The drastic drop from 9 to 12 mo in 20SPFD in Figure

4.3 played a significant role in the deviation because 4 and 37SPFD degradation curves had a much lower margin of error. Overall, it took only 3,4 mo to get an RAvg less than 15% for SPTP, and 9,12 mo for SPFD. The goal margin of error for all foods was < 15% for RAvg and RSD.

The predictive degradation curves for 20STTP had its lowest RAvg at 6,8 mo at 6.6%, and all residual standard deviations were less than 10.1%. 20STTP predictions overwhelmingly overpredicted for all prediction sets. 20STFD had a higher RAvg than 20STTP with many of the predictive sets being over 15% for RAvg and RSD. The overpredictions were also significantly favored when predicting. In summary, 6,8 mo met goal margin of error for STTP, and STFD never met expectations with 1 year of predictive data. However, 12,12 mo data set was close at 15.1% RAvg and 13.1% RSD.

20RATP degradation curves had less than 12% RAvg for all predictive sets post 3,4 mo, and less than 14% RSD, and 20RAFD had much higher error with lowest RAvg and RSD at 14.9% and 13.2%, respectively. All 20RA predictive sets overpredicted. On the other hand, 20RApH3TP had a unique situation where the RAvg was better for TP than FD, which was not the case for the other food groups. Goal error was achieved at 3,4 mo for 20RApH3FD and 12,12 mo for 20RApH3TP. 6,8 mo for 20RApH4 was needed to meet goal standard for RApH4FD, and it was never achieved for RApH4TP. The closest RAvg was 20.6% and all RSDs were above 10%. All RA groups had an overpredicted trend.

Overall, the later the data points during the storage study, the better the RAvg will be. However, it is unreasonable to do prolonged storage studies to get reliable degradation parameters to make predictions because it defeats the purpose of the model.

Thus, the importance in finding a balance of managing storage time versus accuracy of predictions to control time, money, and resources. With using first order kinetics and the exponential temperature dependent rate equation, 12,12 mo predictive set was the best time frame to accommodate all samples at each temperature to get the majority of food less than 15% RAvg and RSD using the current method of averaging the k_{Tref} and c values of 3 different experimental pairs from three different temperature (4, 20, and 37 ^{o}C).

However, the predictions could be improved. First, not all food needed the full 12 months to get a RAvg and RSD less than 15%, so specifically targeting food can reduced storage study. Additionally, the model does not require different storage temperatures to create degradation parameters. If the user knows that the food will remain at a set storage temperature than only doing a storage study at that temperature and determine k_{Tref} and c values at different time point can also reduce storage time, but this can be challenging with fixed order kinetics if the compound is not following a fixed order. Only using 4 and 20 ^{o}C pair also helped because it eliminated the extreme 37 ^{o}C profile that caused many overpredictions of the degradation.

4.4.4. Combined first order kinetics predictions

Another approach was to use the combined first order kinetics model to get better predictions. In Figures 4.3-4.7, there is a degradation curve marked C_{asymp} data. The C_{asymp} data represents the predictions using the combined first order kinetics model. Incorporating this model showed significance ($p \leq 0.05$) for 7 out of the 10 foods, and the outliers where SPTP, SPFD, and RApH4FD. $C_{asymptote}$ is the significant variable that allows this model to be effective. In many cases the residual value of vit C is nonzero.

The combined fixed order kinetic equation allows more flexibility with adjusting the residual value. All $C_{asymptote}$ values for each food, process, and temperature can be viewed in Table 4.1, and the corresponding k_{Tref} and c values can be viewed in Table 4.2.

On another note, there is a caveat in using this approach because $C_{asymptote}$ is only valuable in a distinct period. Longer storage studies are needed to get dependable $C_{asymptote}$ values. This circumstance led to the development of another approach that utilized the data created to make a database that can create degradation parameters with general physicochemical properties of food, such as pH, water activity (Aw), and/or moisture content (%), which will be discussed in the next section.

Table 4.1 C_{asymp} and k(T) values computed in RStudio utilizing all data points with the combined first order kinetics model.

Food	Process	Temp	$C_{asymptote}$	k(T)
SP	TP	4	0.0566	0.006***
SP	TP	20	0.031	0.008***
SP	TP	37	0.0144	0.008***
SP	FD	4	0.2439	0.001***
SP	FD	20	-0.2508	0.002***
SP	FD	37	0.0259	0.017***
ST	TP	4	0.4773**	0.007***
ST	TP	20	0.2807**	0.005***
ST	TP	37	0.0326**	0.011***
ST	FD	4	0.8945***	0.018*
ST	FD	20	0.7781***	0.009*
ST	FD	37	-0.0107***	0.011*
RA	TP	4	0.5089*	0.013***
RA	TP	20	0.3532*	0.007***
RA	TP	37	0.0479*	0.007***
RA	FD	4	0.8737***	0.014**
RA	FD	20	0.6649***	0.004**
RA	FD	37	0.0228***	0.015**
RApH3	TP	4	0.4956***	0.007***
RApH3	TP	20	0.5419***	0.011***
RApH3	TP	37	0.0619***	0.009***
RApH3	FD	4	0.9451***	0.008
RApH3	FD	20	0.9084***	0.013

RApH3	FD	37	-0.0338***	0.007
RApH3	FD	4	0.8655***	0.002
RApH3	FD	20	0.9262***	0.012
RApH3	FD	37	-0.0338***	0.007
RApH4	TP	4	0.3496*	0.01***
RApH4	TP	20	0.4002*	0.012***
RApH4	TP	37	-0.0262*	0.007***
RApH4	FD	4	-0.2102	0.001**
RApH4	FD	20	0.1782	0.003**
RApH4	FD	37	0.0256	0.014**

Table 4.2. k_{Tref} and c values computed in RStudio utilizing all data points with the combined first order kinetics model.

Food	Process	Temps	Time	kTref	c
SP	TP	4, 20, 37	All	0.0075*	0.009
SP	FD	4, 20, 37	All	0.0043	0.116*
ST	TP	4, 20, 37	All	0.008	0.02
ST	FD	4, 20, 37	All	0.0111	-0.019
RA	TP	4, 20, 37	All	0.0078	-0.024
RA	FD	4, 20, 37	All	0.0113	0.002
RApH3	TP	4, 20, 37	All	0.0092	0.007
RApH3	FD	4, 20, 37	All	0.0091	-0.004
RApH3	FD	4, 20, 37	All	0.0073	0.014
RApH4	TP	4, 20, 37	All	0.009	-0.008
RApH4	FD	4, 20, 37	All	0.0043***	0.098***

4.4.5. Categorizing food physiochemical properties to make predictions

To create a database that allows users to insert general physiochemical properties about the food to get degradation parameters, moisture content (MC), water activity (A_w), and pH were determined at the beginning of the storage study, which can be viewed in Table 4.3. Plots for pH and k_{Tref} along with pH and c separated by process (i.e. FD or TP) were created, and linear equations were used to determine pH relationship with degradation parameters. The values were adjusted for MC or Aw by assuming that all FD and TP foods had one distinct MC or A_w value, respectively, Referencing MC, the MC

value was the average of all the FD foods and TP foods, separately. Linear interpolation was used to adjust the c and k_{Tref} values when MC changes.

Table 4.3. List of physiochemical properties for each food and process.

Food	Process	MC (%)	Aw	pH
ST	TP	80.88	0.99	3.50
ST	FD	10.32	0.20	3.50
SP	TP	88.20	0.99	6.20
SP	FD	13.51	0.21	6.20
RApH4	TP	86.12	0.99	3.63
RApH4	FD	11.22	0.09	3.63
RApH3	TP	82.90	0.99	2.93
RApH3	FD	11.92	0.10	2.93
RA	TP	87.26	0.99	3.13
RA	FD	13.86	0.12	3.13

The TP foods pH and k_{Tref} plot (slope: -0.0003, intercept: 0.0096, and R^2: 0.3447) and FD foods pH and k_{Tref} plot (slope: -0.0015, intercept: 0.0135, and R^2: 0.3343) both had a low coefficient of determination. The TP foods pH and c plot (slope: 0.0337, intercept: -0.0135, and R^2: 0.0817) and FD foods pH and c plot (slope: 0.0336, intercept: -0.0882, and R^2: 0.5428) also had low coefficient of determination. C_{asymp} was determined in a similar fashion by creating three C_{asymp}/pH temperature plots at 4 (TP: slope: -0.1398, intercept: 0.9197, and R^2: 0.9513; FD: slope: -0.1692, intercept: 0.2034, and R^2: 0.2034), 20 (TP: slope: -0.1292, intercept: 0.8223, and R^2: 0.83; FD: slope: -0.3255, intercept: 1.7216, and R^2: 0.7907), and 37 ^{o}C (TP: slope: -0.0095, intercept: 0.0629, and R^2: 0.1357; FD: slope: 0.0103, intercept: -0.0341, and R^2: 0.2564). Linear equations and interpolations were utilized to connect C_{asymp}, pH, and moisture content. To adjust for temperature, a C_{asymp}/temperature plot (slope: -0.0031, intercept: 0.085, and R^2: 0.2946) was created with each of the C_{asymp} values determined from the previous plots.

With this data, the user could input food physiochemical property measurements and retrieve degradation parameters based on the two-year experimental storage study data. The user should utilize equation 8 of the combined first order kinetics model to make predictions. The user should also be informed that this model is not perfect because the determination of coefficient was not high for many of the trendlines making this database an unreliable tool to estimate degradation parameters based on general physiochemical properties. This was crucial for C_{asymp} considering its high significance in many of the foods. To improve this, better interpolating functions could be utilized, or segmenting data by using multiple pH ranges could also be implemented; however, more pH values or foods at different pH values should be tested.

On another note, using the endpoints method degradation parameters has more room for error in database infrastructure while still giving reliable predictions. Due to C_{asymp} significance in most foods, especially freeze dry foods, it caused extreme fluctuations in making predictions with physiochemical property data due to low coefficient of determination in most C_{asymp} plots. However, the endpoints method only needs two degradation variables, k_{Tref} and c. The most significant k_{Tref} value has a high coefficient of determination making the predictions much more reliable when inputting food physicochemical properties. 4 and 20 ^{o}C final endpoints were used due to having the lowest RAvg and RSD for 20 ^{o}C data. The storage temperature is not needed for the endpoints method database; however, the user should be mindful that the best results will most likely occur when the storage temperature is between 4 and 20 ^{o}C. Reference Table 4.4 for degradation parameters. To build database, pH/kTref (TP: slope: 0.0014, intercept: -0.0032, and R^2: 0.8467; FD: slope: 0.0013, intercept: -0.0033, and R^2: 0.8175)

and pH/c plots (TP: slope: -0.0011, intercept: 0.0158, and R^2: 0.0097; FD: slope: -0.0029, intercept: 0.0694, and R^2: 0.0084) were constructed with 4 and 20 C final endpoint method results and food physiochemical property data. Unlike the combined first order kinetics plots, the 4 and 20 ^{o}C endpoints method plots had a higher coefficient of determination for its most significant variable, k_{Tref}. This significantly improved degradation parameter predictions; although, this model was less accurate in fitting experimental data. The user interface was like the combined first order kinetics interface except the storage temperature is not needed for the input and the output will not include C_{asymp}.

Table 4.4. 4 and 20 ^{o}C two year endpoints degradation parameters.

Food	Temp	Time	Temp	Time	Process	Order	kTref	c
SP	4	600	20	540	TP	1	0.0060	0.025
ST	4	720	20	720	TP	1	0.0012	0.013
RA	4	720	20	720	TP	1	0.0025	0.045
RApH3	4	720	20	720	TP	1	0.0012	0.010
RApH4	4	720	20	720	TP	1	0.0012	0.008
SP	4	720	20	720	FD	1	0.0045	0.054
ST	4	720	20	720	FD	1	0.0002	0.001
RA	4	720	20	720	FD	1	0.0006	0.035
RApH3	4	720	20	720	FD	1	0.0003	0.100
RApH4	4	720	20	720	FD	1	0.0026	0.100

4.5. Conclusions

Vitamin C (Vit C) is a sensitive compound that is altered by a plethora of factors, which is observed from the vit C loss during the study under different conditions. Based on our findings, the endpoints method was able to approximately provide less than 15% residual average and standard deviation difference for all foods at 12 month timepoints using average k_{Tref} and c pairs at all temperatures. When 37 ^{o}C was removed and only 4 and 20 ^{o}C pair was used, the residual average improved further improved for all foods

except 20SPFD. It also further improved with only using 20 ^{o}C and adjusting the timepoints; however, this had its limitations when using only fixed-first order kinetics.

The combined fixed-first order kinetics model played a crucial role in improving degradation parameters for the stable vit C foods that had a non-zero residual value. To further utilized this data, a database was built to give users rough estimates of degradation parameters based on general physiochemical properties. This tool can be very resourceful for individuals who are not able to conduct their own storage study to find degradation parameters. However, the database needed more exact interpolating functions to consistently work well. Due to this, a database using the endpoints method with final degradation parameters at 4 and 20 ^{o}C was also developed. This setup provided more reliable degradation parameters when using linear trendlines to connect food physiochemical properties with degradation parameters.

Moreover, the low acid food, such as sugar snap peas, did the best at following fixed first order kinetics and worked very well with the endpoints method model. This was also noticed in the higher acidic pH foods such as RApH4FD. Essentially, pH $\geq$ 3.63 had a higher chance of following first order kinetics. On the other hands, foods $\leq$ 3.13 fitted the combined first order kinetics model much better due to the nonzero residual asymptote, which makes logical sense considering vit C is much more stable in an acidic environment, which we also have confidence this help reduce its aerobic degradation.

Overall, if a team wanted to ensure astronauts are getting all the necessary vit C for shelf stable foods at any point during space, then preparing a freeze-dried food in an acidic medium is the best approach to take because many of the freeze dried acidic foods had little to no degradation during the storage study. Vit C is also in higher abundance in

the freeze dried foods compared to retort thermoprocessing, which wipes out so much of the vit C content. This was highly noticeable in sugar snap peas.

CHAPTER 5

CONCLUSION

The body of work has outlined a variety of approaches to model vitamin C degradation during retort thermal processing and long-term storage. During the retort thermal processing study, we found that fixed order kinetics combined with the nonisothermal endpoints methods can be a resourceful tool for monitoring vitamin C during processing and help control vitamin C during food preservation. We also found that fixed second order kinetics in rhubarb applesauce had an overall lower residual average than fixed first order kinetics; however, fixed first order kinetics had a better residual average in sugar snap peas, but the margin of improvement from fixed second order kinetics was tenuous. During the long-term storage study, we found that fixed order kinetics was not good enough by itself, and it was necessary to use the combined fixed order kinetics model to make predictions. This approach helped overcome the overprediction of those non-zero residual foods from first order kinetics alone. From this, we also noticed the importance of focusing on freeze dried acidic foods to make sure astronauts are getting all the necessary vitamin C during long-term spaceflight because the low acid and thermal processed foods tends to have minimum vitamin C and degrades more rapidly than freeze dried food near room temperature. If astronauts prefer retorted food more, then changing retort recipe to a rotary recipe providing the same lethality would help reduced vitamin C degradation and potentially prolonged its retention. We were also able to develop a database for degradation parameters based on experimental and physiochemical data that can be a great complementary tool to aid in making predictions.

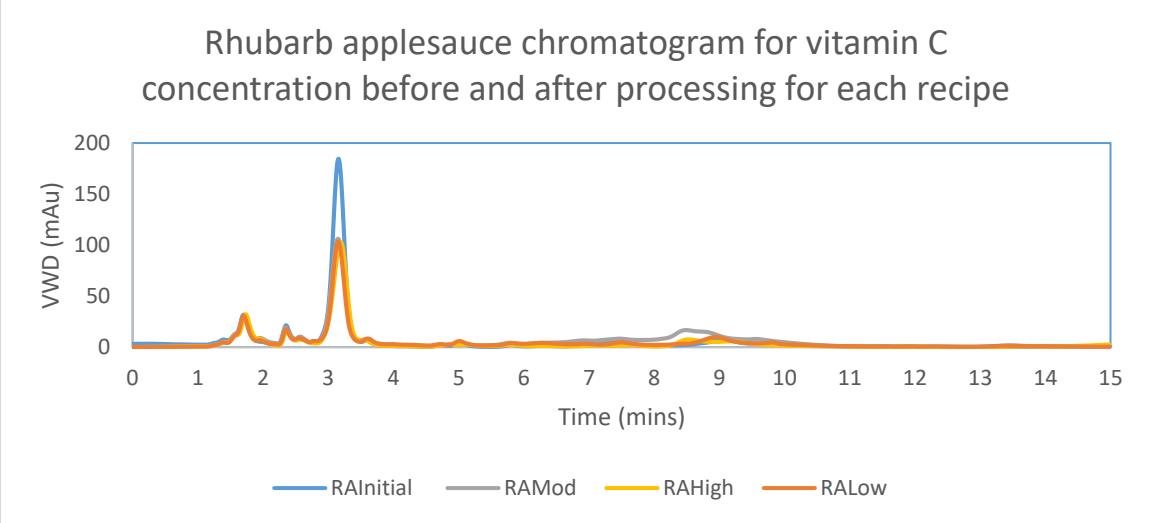
APPENDIX A

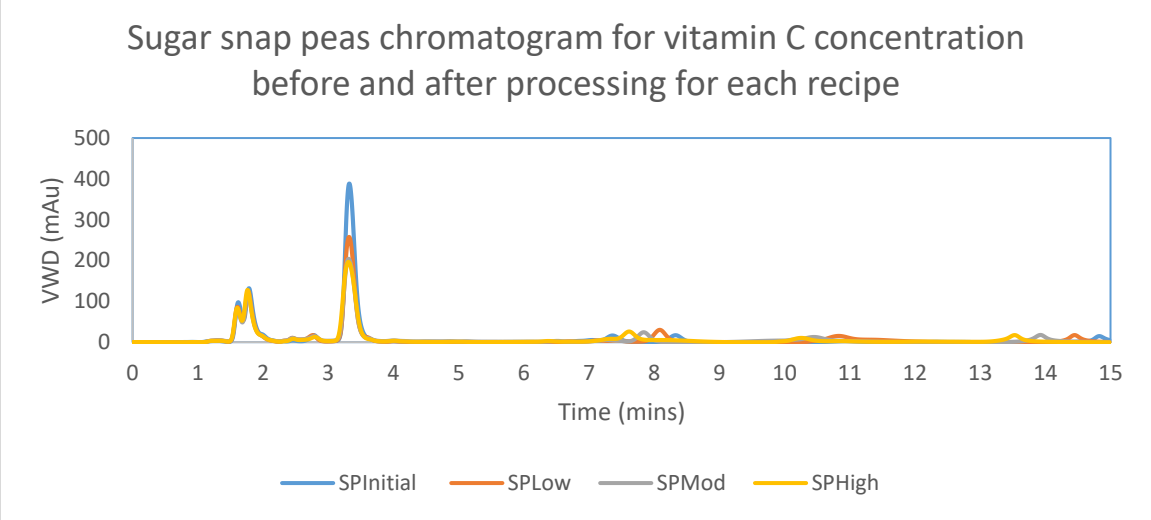
VITAMIN C CONCENTRATION IN SUGAR SNAP PEAS AND RHUBARB APPLESAUCE PRE-AND POSTPROCESSING AT 3 DIFFERENT TEMPERATURE PROFILES.

Name	Conc (mg/100g)	Name	Conc (mg/100g)
RAInitial1	7.30	SPInitial1	25.4
RAInitial2	7.90	SPInitial2	23.1
RAInitial3	8.15	SPInitial3	22.6
RAInitial4	8.08	SPInitial4	23.2
RAInitial5	8.41	SPInitial5	20.9
RAInitial6	7.58	SPInitial6	21.6
RALow1	4.31	SPLow1	17.9
RALow2	4.72	SPLow2	16.3
RALow3	4.70	SPLow3	18.4
RALow4	4.69	SPLow4	18.2
RALow5	4.56	SPLow5	17.6
RALow6	4.56	SPLow6	19.5
RAMod1	4.88	SPMod1	15.1
RAMod2	4.76	SPMod2	16.6
RAMod3	5.40	SPMod3	16.6
RAMod4	5.22	SPMod4	16.4
RAMod5	5.01	SPMod5	19.8
RAMod6	4.86	SPMod6	18.1
RAHigh1	4.85	SPHigh1	16.1
RAHigh2	5.29	SPHigh2	17.6
RAHigh3	5.61	SPHigh3	18.5
RAHigh4	5.90	SPHigh4	17.2
RAHigh5	5.48	SPHigh5	18.4
RAHigh6	5.32	SPHigh6	19.7

APPENDIX B

RHUBARB APPLESAUCE AND SUGAR SNAP PEAS CHROMATOGRAMS FOR VIT C PRE-AND POST RETORT THERMAL PROCESSING AT THREE TEMPERATURE PROFILES (LOW, MOD, AND HIGH)

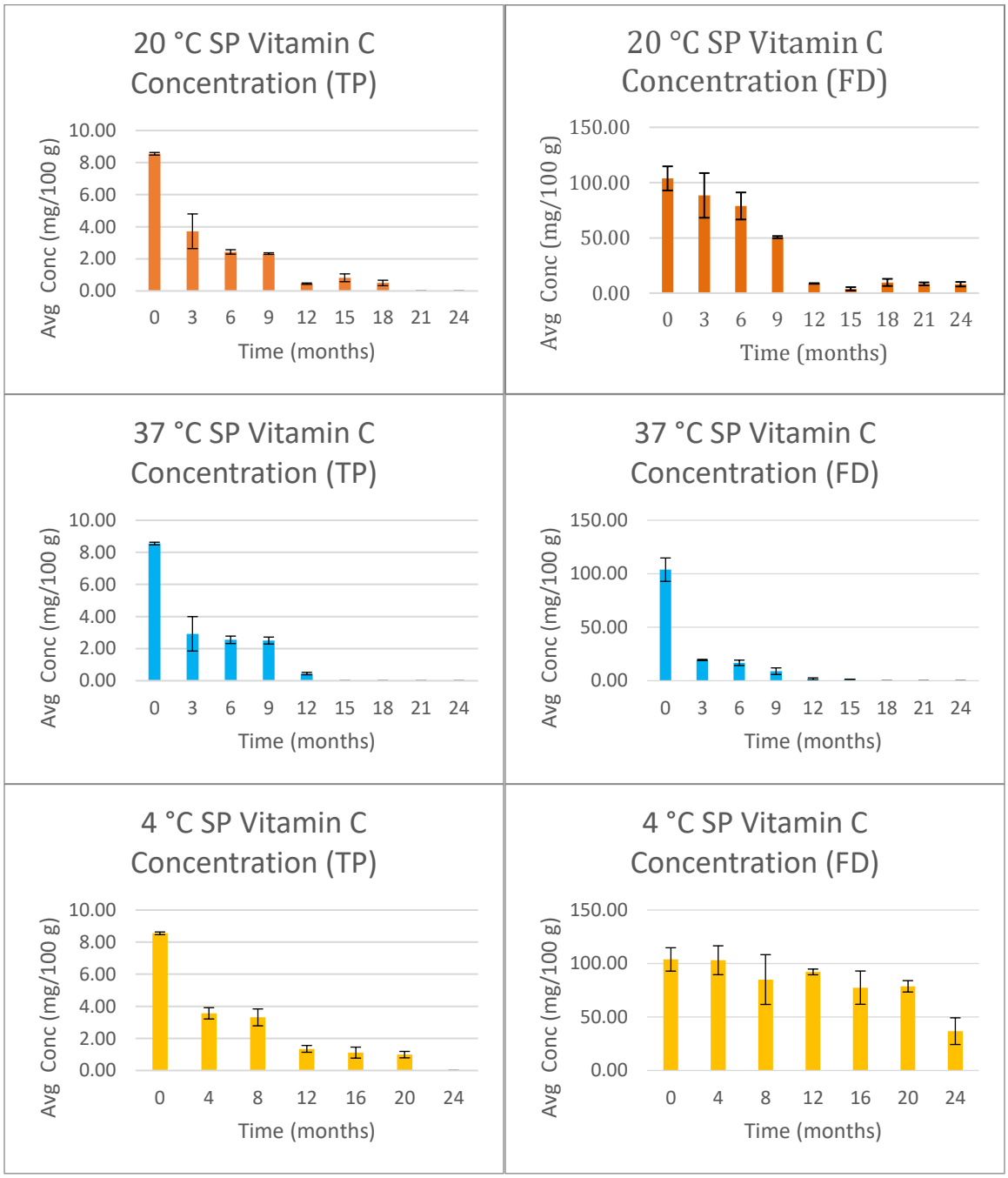


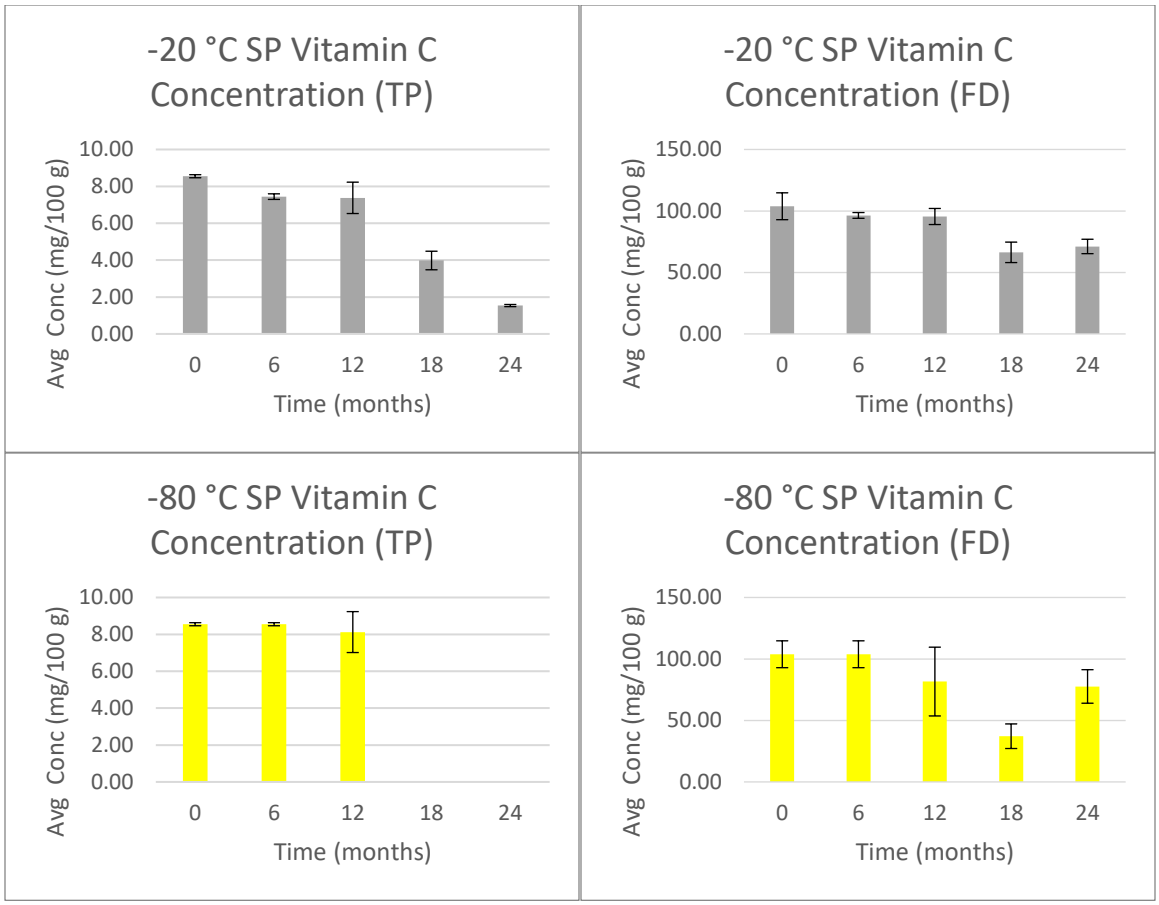


APPENDIX C

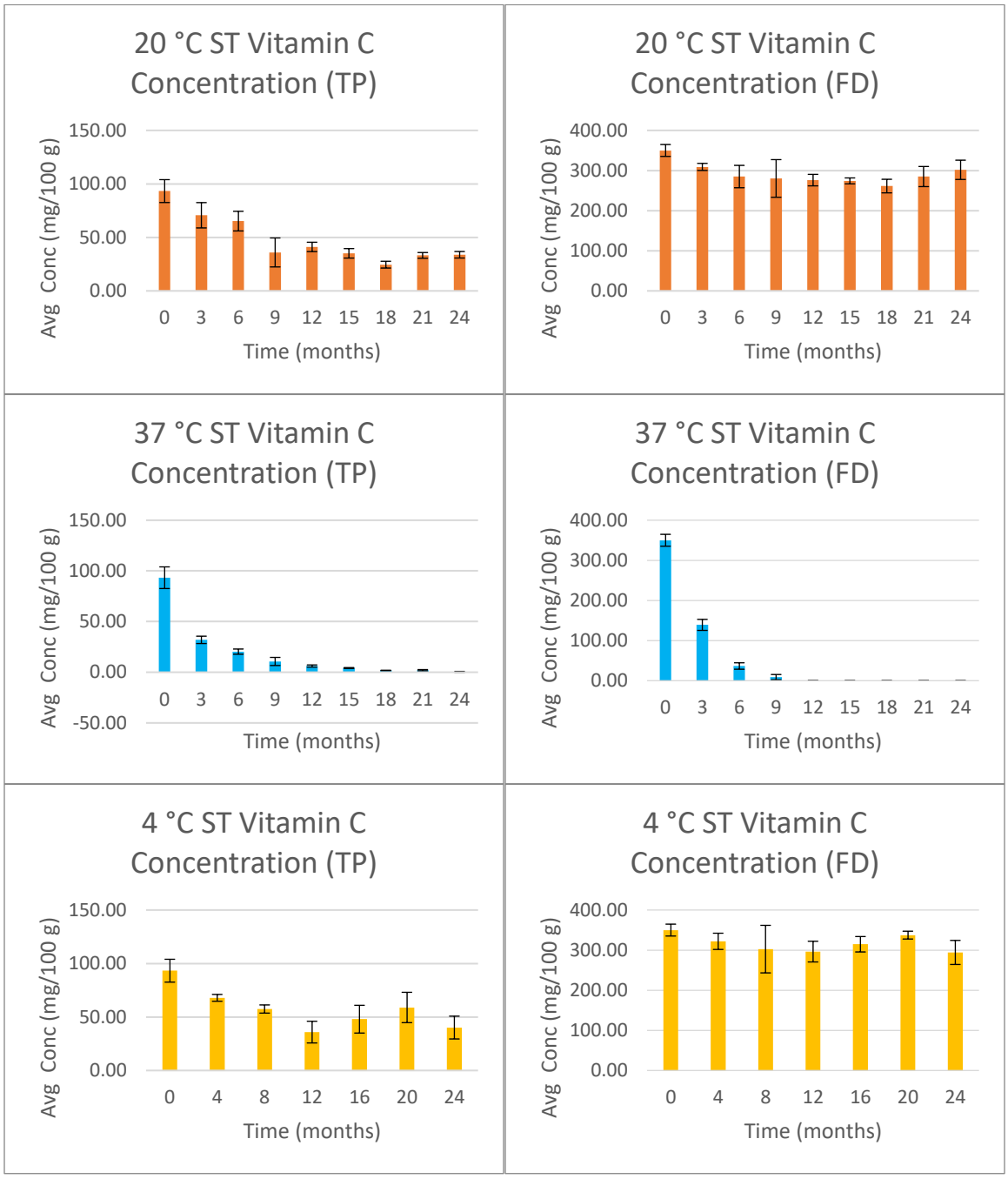
VITAMIN C CONCENTRATION FOR SUGAR SNAP PEAS, STRAWBERRIES, RHUBARB APPLESAUCE, RHUBARB APPLESAUCE PH 3, AND RHUBARB APPLESAUCE PH4 AT 4, 20, 37, -20, AND -80 ºC STORAGE TEMPERATURES.

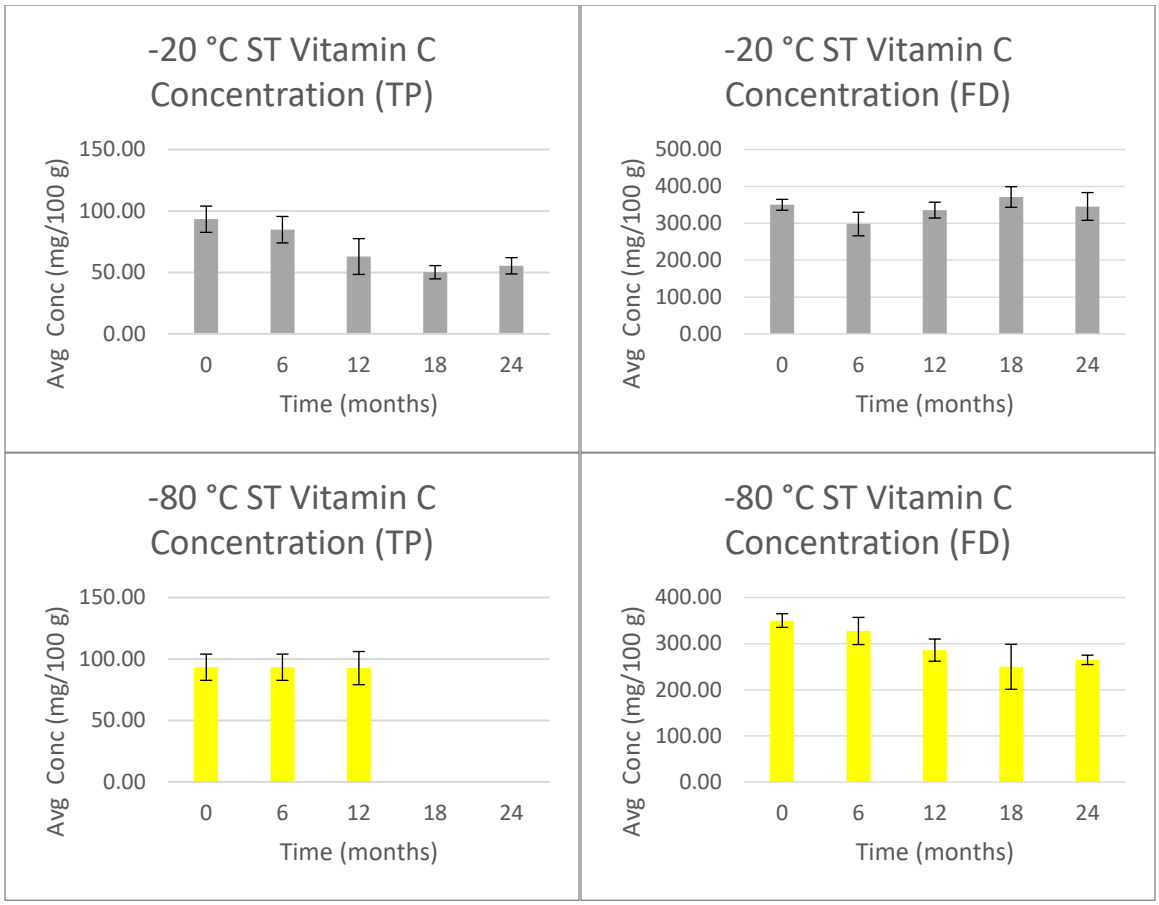
Sugar Snap Peas



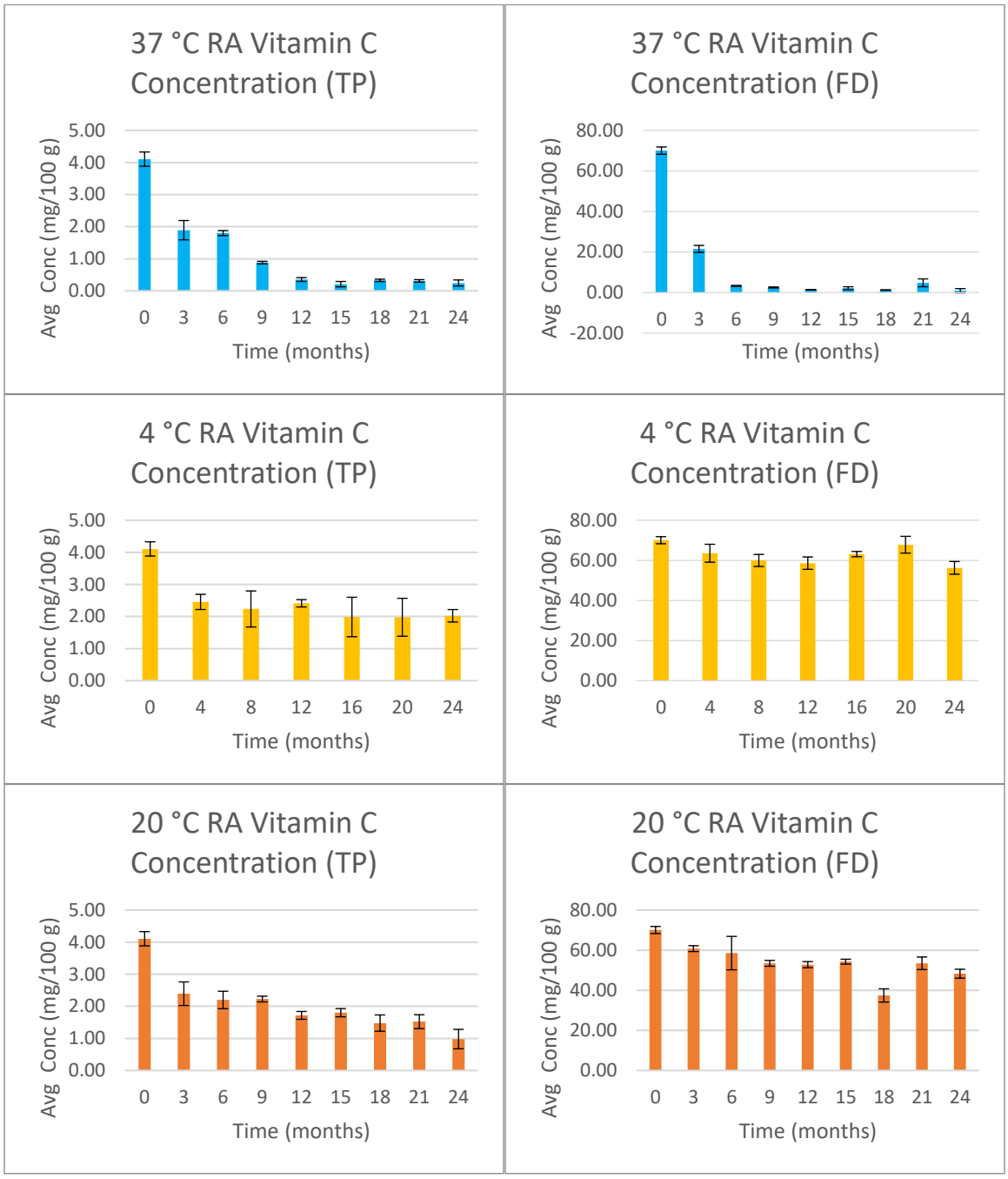

-20 °C SP Vitamin C Concentration (TP)
Avg Conc (mg/100 g)
10.00
8.00
6.00
4.00
2.00
0.00
0
6
12
18
24
Time (months)
-20 °C SP Vitamin C Concentration (FD)
Avg Conc (mg/100 g)
150.00
100.00
50.00
0.00
0
6
12
18
24
Time (months)
-80 °C SP Vitamin C Concentration (TP)
Avg Conc (mg/100 g)
10.00
8.00
6.00
4.00
2.00
0.00
0
6
12
18
24
Time (months)
-80 °C SP Vitamin C Concentration (FD)
Avg Conc (mg/100 g)
150.00
100.00
50.00
0.00
0
6
12
18
24
Time (months)

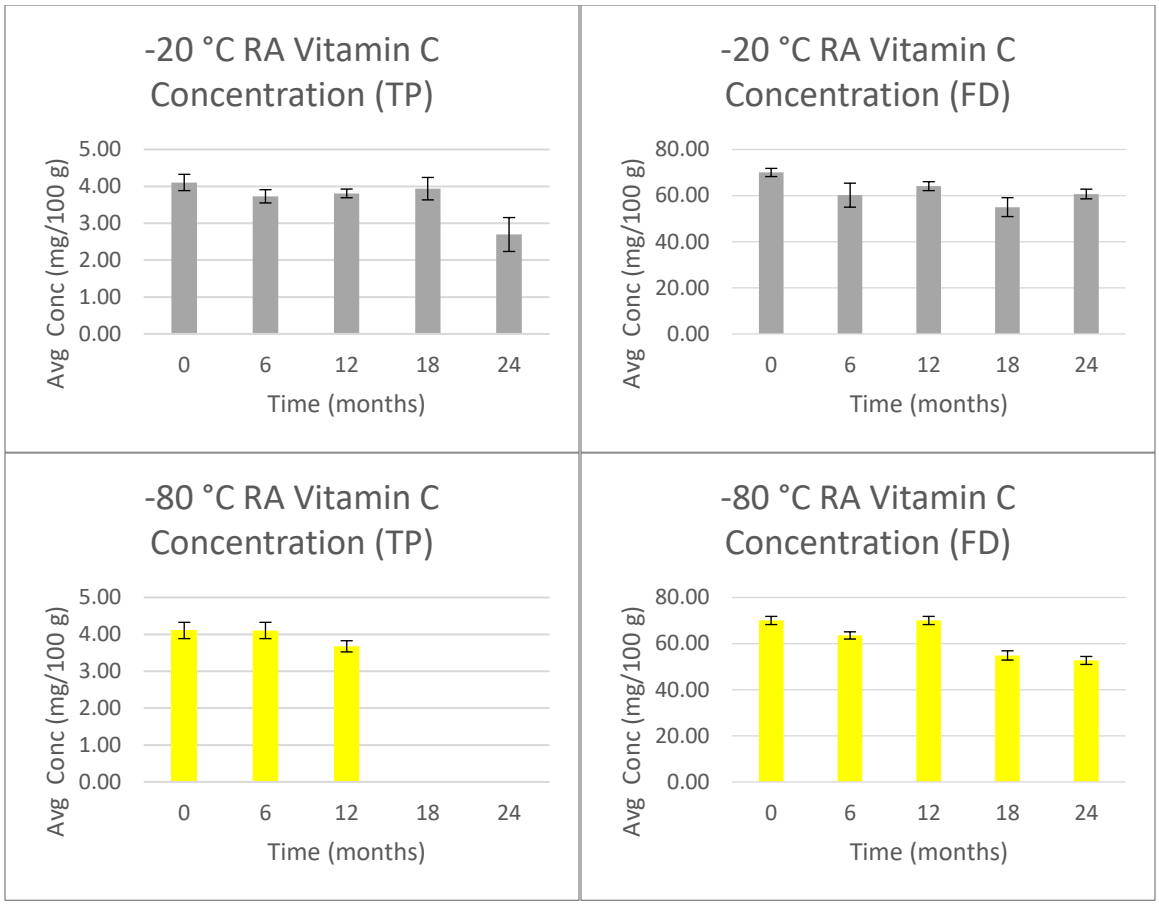
Strawberries




-20 °C ST Vitamin C Concentration (TP)
Avg Conc (mg/100 g)
150.00
100.00
50.00
0.00
0
6
12
18
24
Time (months)
-20 °C ST Vitamin C Concentration (FD)
Avg Conc (mg/100 g)
500.00
400.00
300.00
200.00
100.00
0.00
0
6
12
18
24
Time (months)
-80 °C ST Vitamin C Concentration (TP)
Avg Conc (mg/100 g)
150.00
100.00
50.00
0.00
0
6
12
18
24
Time (months)
-80 °C ST Vitamin C Concentration (FD)
Avg Conc (mg/100 g)
400.00
300.00
200.00
100.00
0.00
0
6
12
18
24
Time (months)

Rhubarb Applesauce




-20 °C RA Vitamin C Concentration (TP)
Avg Conc (mg/100 g)
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4.00
3.00
2.00
1.00
0.00
0
6
12
18
24
Time (months)
-20 °C RA Vitamin C Concentration (FD)
Avg Conc (mg/100 g)
80.00
60.00
40.00
20.00
0.00
0
6
12
18
24
Time (months)
-80 °C RA Vitamin C Concentration (TP)
Avg Conc (mg/100 g)
5.00
4.00
3.00
2.00
1.00
0.00
0
6
12
18
24
Time (months)
-80 °C RA Vitamin C Concentration (FD)
Avg Conc (mg/100 g)
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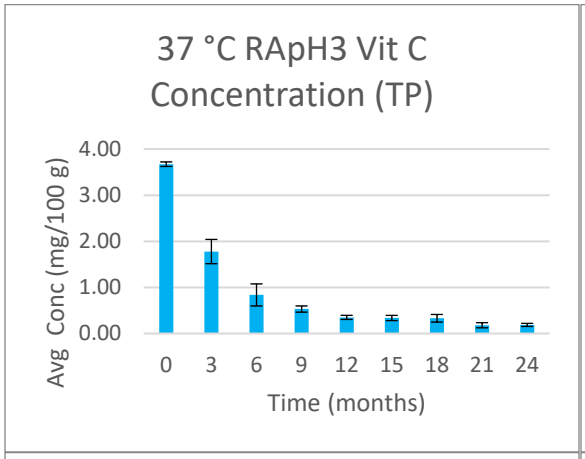
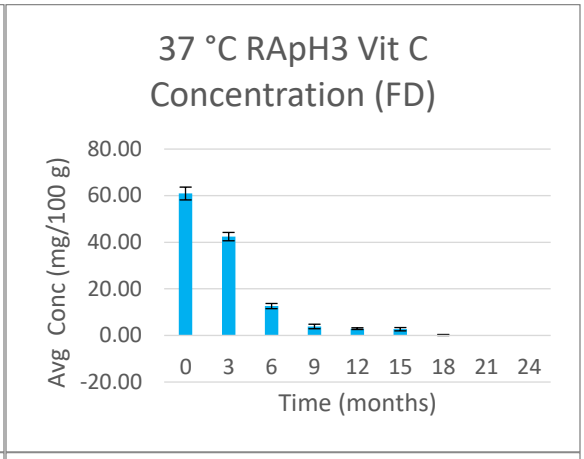
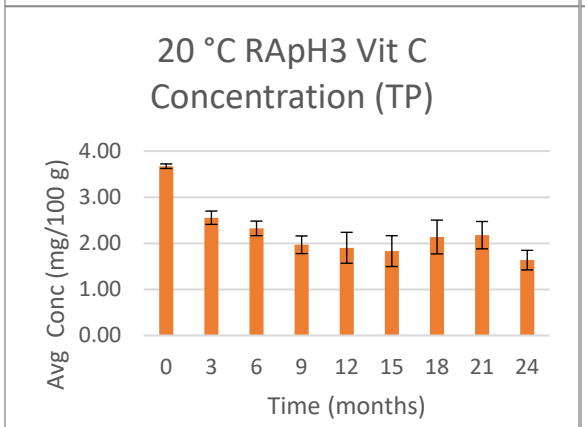
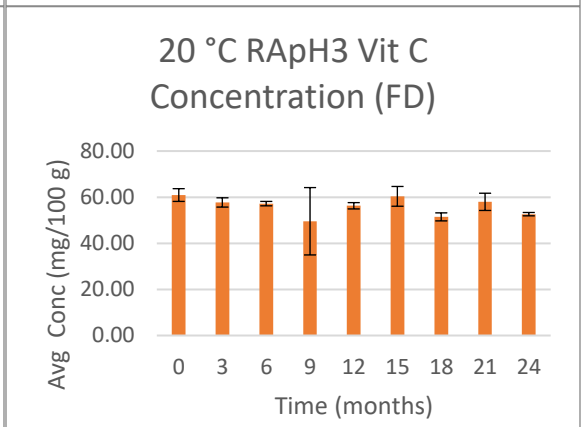
60.00
40.00
20.00
0.00
0
6
12
18
24
Time (months)

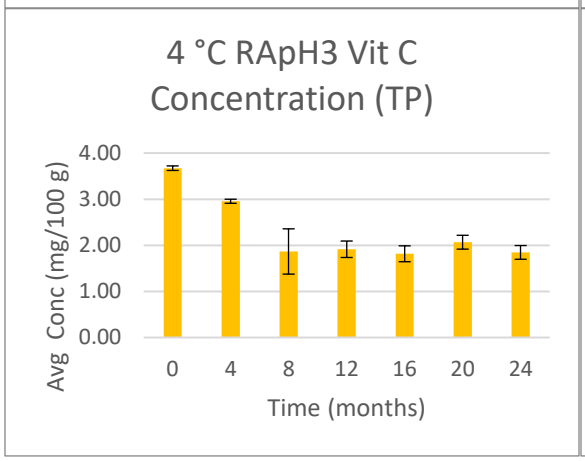
Rhubarb Applesauce pH3

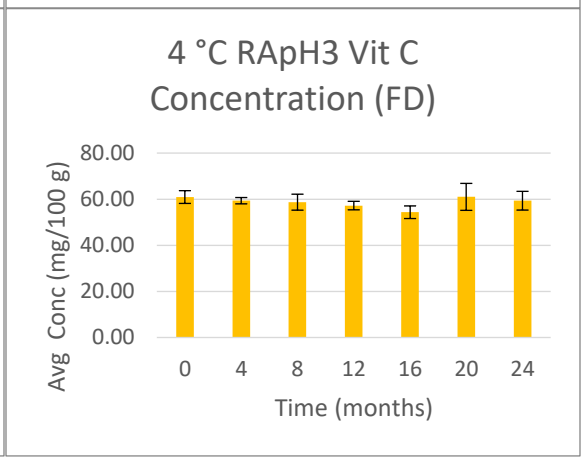


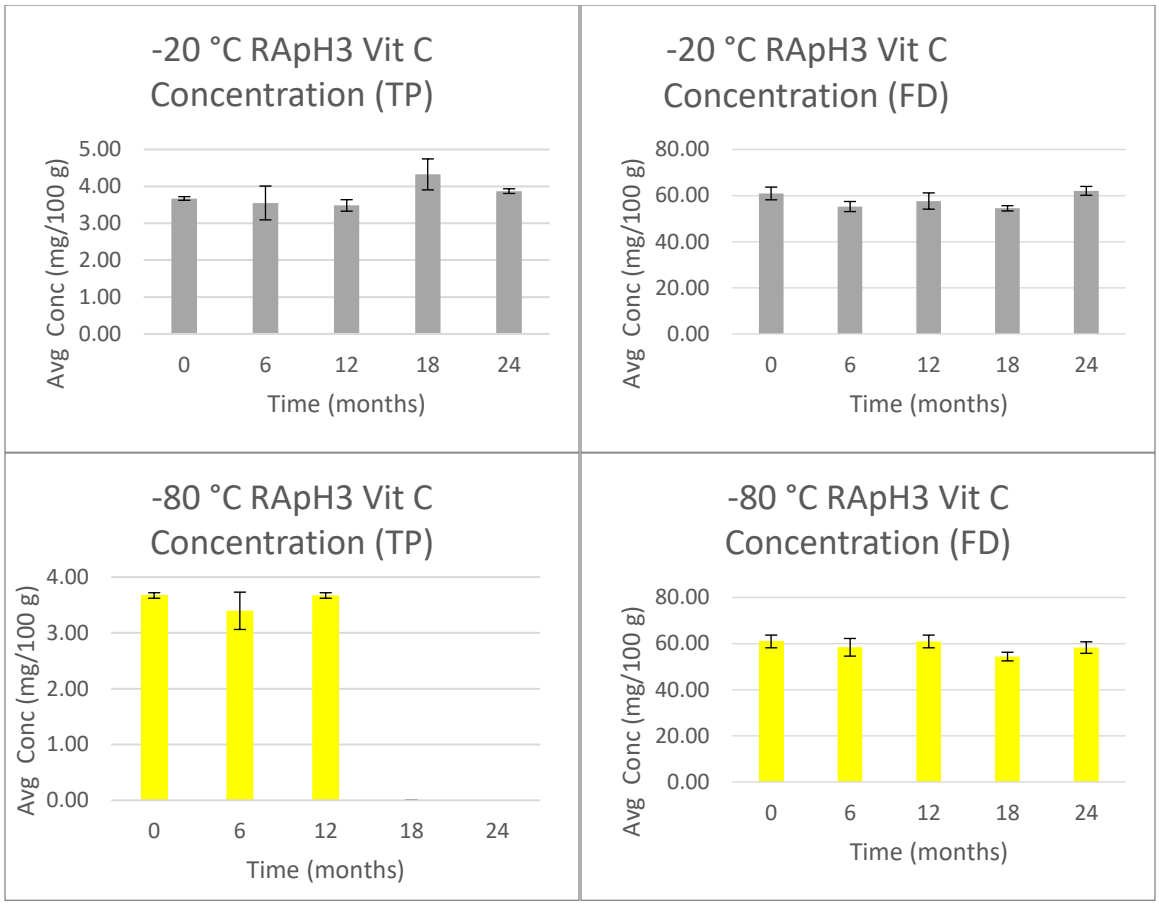








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Avg Conc (mg/100 g)
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4.00
3.00
2.00
1.00
0.00
0
6
12
18
24
Time (months)
-20 °C RApH3 Vit C Concentration (FD)
Avg Conc (mg/100 g)
80.00
60.00
40.00
20.00
0.00
0
6
12
18
24
Time (months)
-80 °C RApH3 Vit C Concentration (TP)
Avg Conc (mg/100 g)
4.00
3.00
2.00
1.00
0.00
0
6
12
18
24
Time (months)
-80 °C RApH3 Vit C Concentration (FD)
Avg Conc (mg/100 g)
80.00
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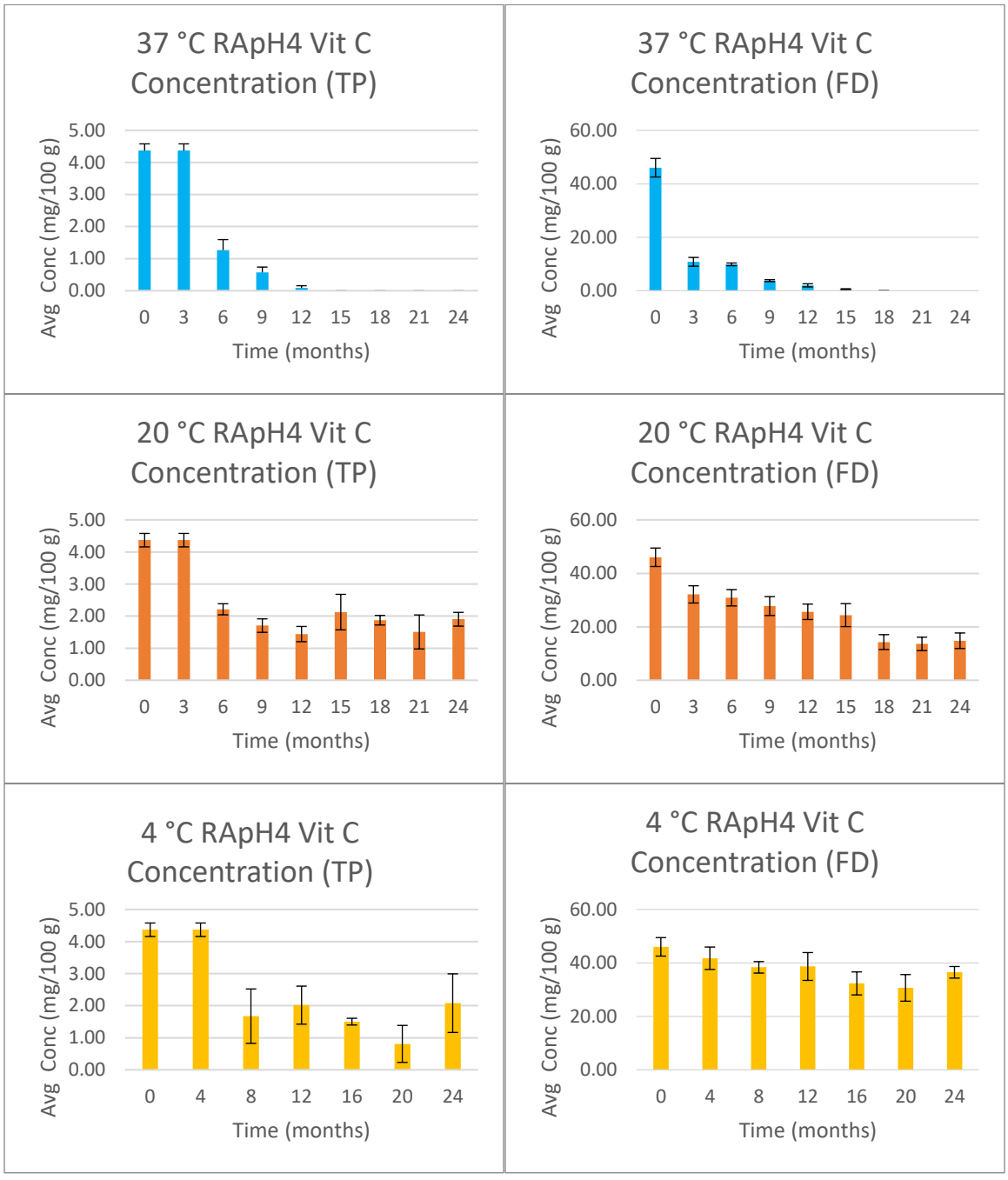
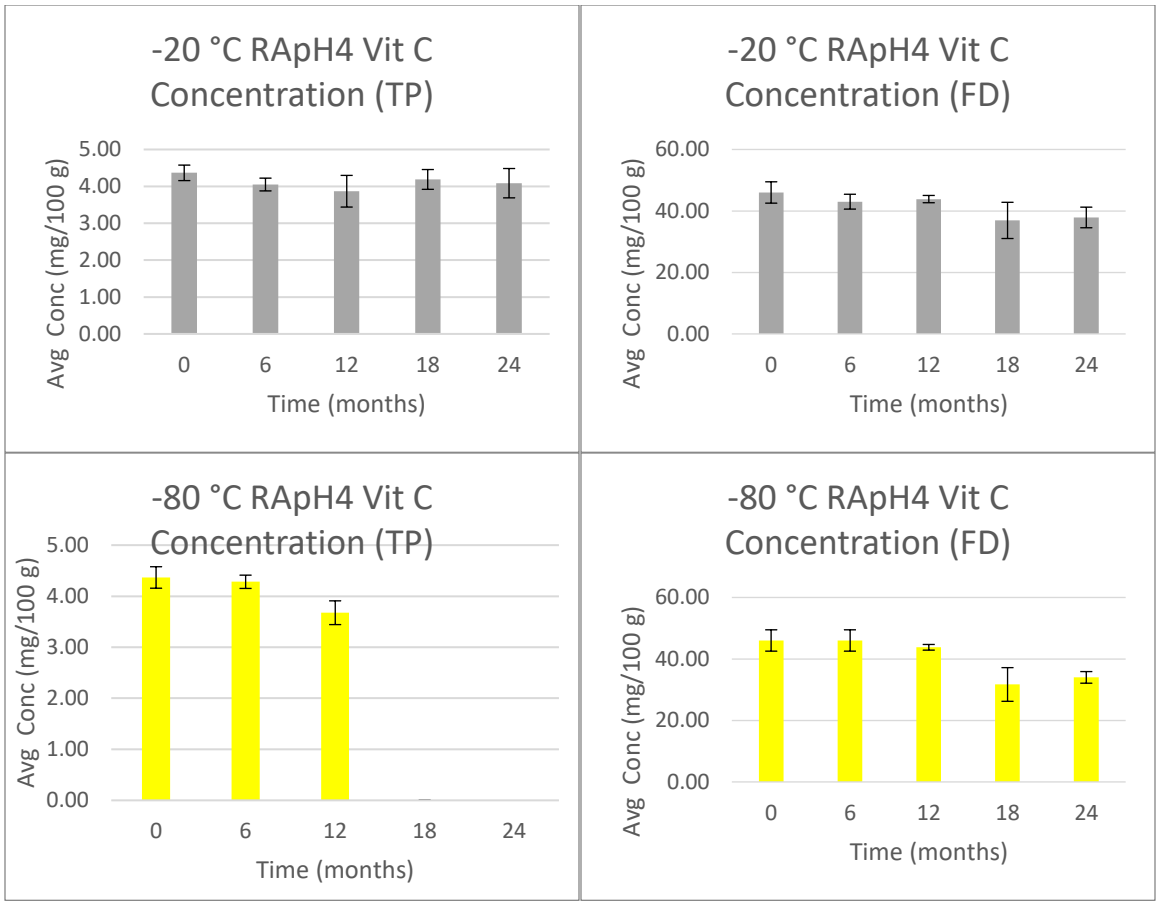
40.00
20.00
0.00
0
6
12
18
24
Time (months)

Rhubarb Applesauce pH4

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1.00
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0
6
12
18
24
Time (months)
-20 °C RApH4 Vit C Concentration (FD)
Avg Conc (mg/100 g)
60.00
40.00
20.00
0.00
0
6
12
18
24
Time (months)
-80 °C RApH4 Vit C Concentration (TP)
Avg Conc (mg/100 g)
5.00
4.00
3.00
2.00
1.00
0.00
0
6
12
18
24
Time (months)
-80 °C RApH4 Vit C Concentration (FD)
Avg Conc (mg/100 g)
60.00
40.00
20.00
0.00
0
6
12
18
24
Time (months)

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