



Kinetic Modeling of Vitamin C Degradation in Lettuce (*Lactuca sativa L*) under Room and Cold Temperatures Using Computer Simulation Analysis

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Abstract: This study investigates the kinetic modeling of vitamin C degradation in lettuce under room and cold temperatures of 17.5 °C, 19.5 °C, 21 °C and 6.5 °C, 7.5 °C, and 9.5 °C respectively using computer simulation analysis. High-Performance Liquid Chromatography is employed to assess the vitamin C concentrations in the lettuce samples, utilizing an isocratic elution procedure of flow rate of the mobile phase at 1.2 cm³ min⁻¹ and an injection volume of 20 μL. The temperature of the analytical column is kept constant at 25 °C coupled with ultraviolet-visible detection set at 245 nm. The lettuce kept at room and cold temperatures for nine days show a reduction in vitamin C with increasing temperature and time. The degradation of vitamin C followed a first-order kinetic model as the average coefficient of determination (*R*²-value) for room and cold temperatures tending to 1: 0.922843 and 0.940793 respectively. The integrated law method of first order kinetics gave rate constants of 0.855, 0.925, 0.991 and 0.497, 0.51, 0.546 k (min⁻¹) for the room and cold temperatures with corresponding half-lives of 0.8107, 0.7493, 0.6994 and 1.3947, 1.3591, 1.2695 days respectively. A mathematical model is created on the computer and the model's behavior is explored by running the simulation (forecast). The predicted kinetic models formulated gives the best prediction at $\ln(C) = \ln(C_0) - 0.497t$. The activated energy (EA) yielded values of 10.2220 and 30.4706 kcal/mol for both temperatures respectively. The experimental and computer simulation analysis indicates that lettuce at 6.5 °C retain higher vitamin C concentration.

Keywords: Vitamin C, lettuce, degradation, modeling, computer simulation analysis.

Submitted: November 04, 2023. **Accepted:** February 18, 2025.

Cite this: Emenike A, Okoroafor C. Kinetic Modeling of Vitamin C Degradation in Lettuce (*Lactuca sativa L*) under Room and Cold Temperatures Using Computer Simulation Analysis. JOTCSA. 2025;12(2):65–76.

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1. INTRODUCTION

Vegetables are rich in carbohydrates and fiber and low in fat and energy. They also include considerable amounts of micronutrients (1). Cultivated Lettuce (*Lactuca sativa L.*) is a widely grown and popularly consumed leafy vegetable because it contains vitamin C, polyphenols, and dietary fiber, which contribute to weight loss (due to its low caloric content), lower the risk of

cardiovascular diseases (via reducing low-density lipoprotein (LDL) cholesterol and blood pressure), and reduce the risk of diabetes (by improving glucose metabolism) and colon cancer (due to protective role of dietary fiber) (2). The major traditional use of lettuce is as a sleep inducer. An investigation into lettuce extracts led to the discovery of a depressive compound. Significant sedative effects were observed in experimental animals when this drug was given to them (3).

quantitatively. Understanding the fundamental reaction mechanisms that are essential for quality modelling and control is another benefit of kinetic modelling. In food systems, the kinetics of ascorbic acid degradation are more complicated, although in model systems, they follow first-order kinetics (24). Since the design of mechanistic models is hampered by the complexity of the degradation mechanisms, pseudo-kinetic models—such as zero-, first-, or second-order kinetics—are frequently used to get an ideal coordinate with the experimental data. The model that gives the highest coefficient of determination value (R^2 value) is regarded as the best fit for the analysis (25).

The simulation technique is the process of designing a model of a real system and conducting experiments with the purpose either of understanding the behavior of the system or evaluating various strategies for the operation of the system. Simulation has also been defined as the broad collection of methods used to study and analyze the behavior and performance of actual or theoretical systems. In simulation analysis, we build a mathematical model of a system, process, or other entity, usually on a computer, and then use a simulation to examine the model's behavior. A time series is a chronological sequence of observations on a particular variable. Usually, the observations are taken at regular intervals (minutes, days, months, years), but the sampling could be irregular. A time series analysis consists of two steps: (1) building a model that represents a time series, and (2) using the model to predict (forecast) future values. The objectives of this study were (i) to determine the rate of degradation of vitamin C in lettuce under pretreatment conditions of room and cold temperatures, to recommend the best; (ii) to develop kinetic models for predicting vitamin C degradation in the lettuce under the studied conditions. (iii) to predict the future values (forecast) (26).

2. MATERIALS AND METHODS

2.1 Reagents and chemicals

L-ascorbic acid (AA), metaphosphoric acid (MPA), orthophosphoric acid, and acetonitrile (HPLC grade) were all purchased from Merck (Darmstadt, Germany). For chromatographic analysis, de-ionized water of $18 \text{ M}\Omega\text{cm}^{-1}$ resistivity purified with a milli-Q system (Millipore, Bedford, USA) was used. The ascorbic acid stock standard solution was prepared in water and stored in a glass-stopper bottle at $4 \text{ }^\circ\text{C}$ in the dark (27).

2.2 Sample preparation

Fresh and matured lettuce was sourced from fruits and vegetable market located in Yankaba market,

Nasarawa local Government of Kano state, Nigeria which lies between Longitude $70 \text{ }^\circ 54'$ and $90 \text{ }^\circ 06'$ East and Latitude $110 \text{ }^\circ 37'$ and $120 \text{ }^\circ 21'$ north. The fresh and matured lettuce vegetable of about 3 kg was washed under running tap water for about 2 min; the stems were removed by cutting with a sharp and pre-washed stainless steel knife to avoid contamination. The leaves of about 100 g each for the six (6) sets of temperatures; $17.5 \text{ }^\circ\text{C}$, $19.5 \text{ }^\circ\text{C}$, and $21 \text{ }^\circ\text{C}$ for room temperatures and $6.5 \text{ }^\circ\text{C}$, $7.5 \text{ }^\circ\text{C}$, and $9.5 \text{ }^\circ\text{C}$ for cold temperatures were made set for the experiment by draining them differently using muslin cloth for 5 min and the initial samples were blended in a Kenwood blender (Philips, HR 1702, Borehamwood, England, UK) and filtered with cheese-cloth. At two-day intervals, the samples were analyzed for vitamin C using high-performance liquid chromatography (HPLC). On the first day, initial unaltered samples maintained at specified room and cold temperature served as control. The remaining lots were spread evenly on trays and dried under room mild temperatures and the other half kept under cold temperatures. The temperature and relative humidity were measured using thermometer and hygrometer respectively. The lettuce vegetable wastes are bio-degradable in nature; they were disposed by putting in the soil where microbes acted on them enriching the soil. The experiments were carried out in 3 replications and the average of measurement was reported (28).

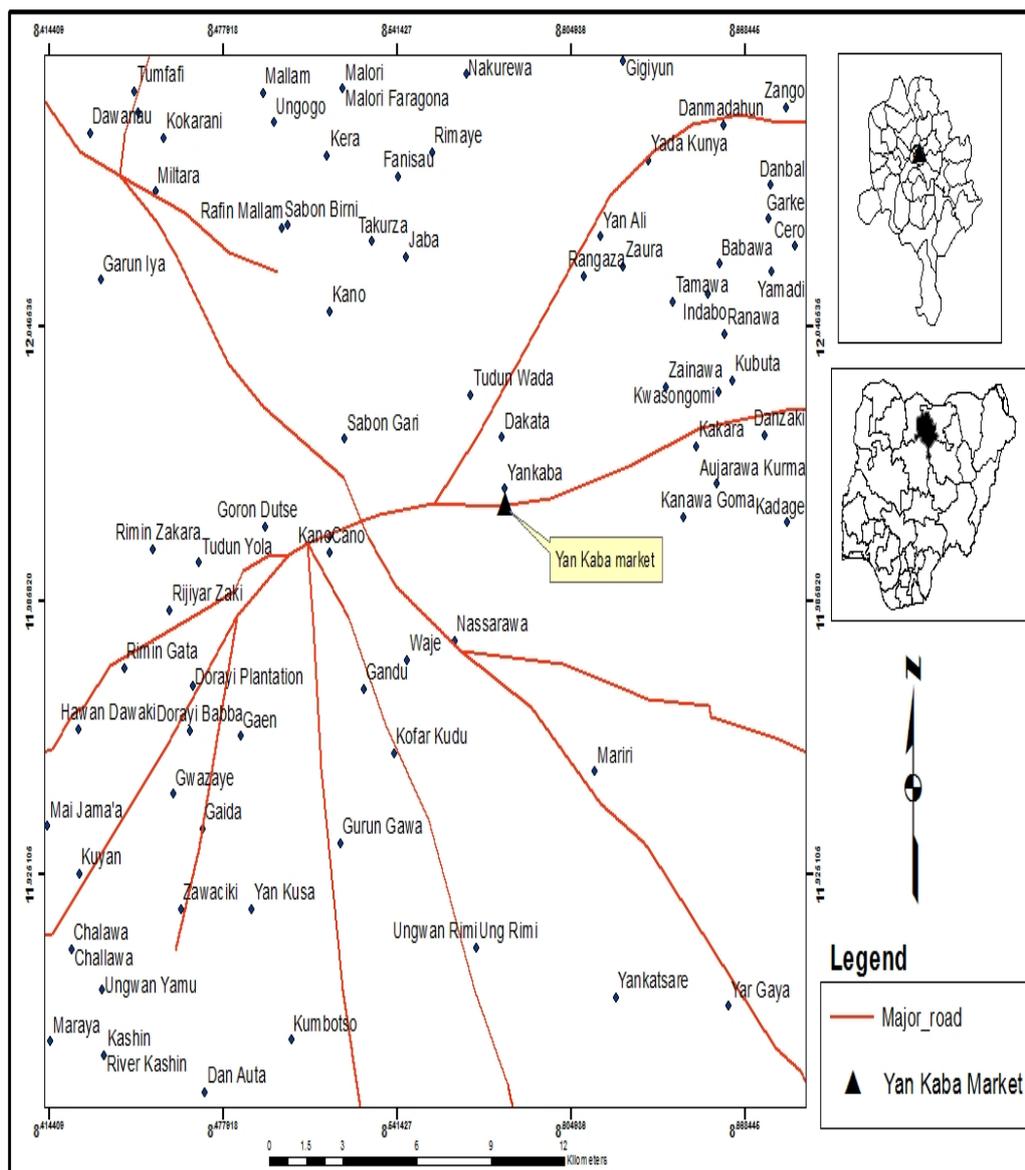
2.3 Instrumentation

The HPLC system consists of Waters liquid chromatography (Milford, MA, USA) equipped with a 600E multisolvent delivery system, an in-line degasser, a manual injection with $20 \text{ }\mu\text{L}$ loop (Rheodyne 7125), and Waters 2487A dual absorbance detector. Empowers software was used for controlling the analytical system and data processing.

2.3.1 Extraction of ascorbic acid

2.3.2. Mild-temperature-drying procedure

This procedure is a modification of Rahman's method (29). About 100 g of salad vegetable samples in each maturity stage were separately weighed and dried under mild temperature ($15\text{--}20 \text{ }^\circ\text{C}$) and ground to fine powder dust before extraction. The obtained powder were weighed (1.0 g for each sample and subsequently extracted with 25 mL of extractant solution, containing 5% MPA, at $10 \text{ }^\circ\text{C}$ and in the dark. The extraction process was performed using a shaker for 4 hours. All extractions were carried out in triplicate and obtained solutions were then filtered and stored at $4 \text{ }^\circ\text{C}$ before analysis. The injection of the extracts into the HPLC system was performed twice.



Source: Data analysis (2018)

2.3.3. HPLC analysis

Ascorbic acid was determined using a liquid chromatographic technique that included an isocratic elution process and UV-visible detection at 245 nm. Separations were carried out on a 5 μm RP C18 column of 250 mm \times 4.6 mm (Spherical, Optimals ODS-H, Capital HPLC, UK) fitted with a 5 μm RP C18 guard column of 20 mm \times 4.6 mm (Spherical, Optimals ODS-H, Capital HPLC, UK). The mobile phase employed was a mixture of 0.5% NaH_2PO_4 (pH 2.25 with H_3PO_4)–acetonitrile (93:7). The Flow rate of the mobile phase was 1.2 mL min^{-1} and an injection volume of 20 μL was used in quantitative analysis. The desired flow rate was set on the HPLC pump, then a known volume

container was used to collect the eluent for a specific time. The flow rate was now calculated by dividing the collected volume by the time elapsed. The temperature of the analytical column was kept constant at 25 $^\circ\text{C}$. The calibration curve and quantitative evaluations were accomplished at 245 nm. Standard solutions and extracts were filtered through a prefilter and then a 0.45 μm Millipore membrane before their injection. To prevent the loss of AA, standard solutions and extracted samples were protected from light using amber flasks. Quantitation was performed by comparing the chromatographic peak area with that of the external standard. The calibration curve was

plotted in the concentration range of 0.5–200 mg L⁻¹ and based on a 10-point calibration (28).

2.4 Kinetic modeling

Using the integrated rate law, the breakdown of vitamin C was modeled. Using the integral approach of analysis, various models were created. As shown in the following integral law equation:

$$\frac{dC}{dt} = -K[C]^n \tag{1}$$

was utilized to create three concentration-based models with corresponding half-lives (t_{1/2}) for reaction orders n = 0, 1, and 2. Zero order model (n = 0):

$$C = C_0 - kt \tag{2a}$$

$$t_{\frac{1}{2}} = \frac{C_0}{2k} \tag{2b}$$

First order model (n = 1):

$$\ln(Ckt) = \ln(C_0) - kt \tag{3a}$$

$$(t_{\frac{1}{2}}) = \ln \frac{(2)}{k} \tag{3b}$$

Second order model (n = 2):

$$\frac{1}{C} = \frac{1}{C_0} + kt \tag{4a}$$

$$t_{\frac{1}{2}} = \frac{1}{kC_0} \tag{4b}$$

where k = rate constant, C₀ = initial concentration of vitamin C in the sample, C = concentration of vitamin C in the sample at time t, and t_{1/2} = half-life of vitamin C in the sample.

2.4.1 Arrhenius equation

$$K = A_p \frac{E_A}{RT} \tag{5a}$$

k = Rate Constant, A = Frequency Factor or Pre-experimental Factor, e = Mathematical quantity (e), R = the gas Constant, T = Kelvin Temperature, E_A = Activation Energy

Arrhenius's equation shows the effect of temperature on the rate constant and therefore on the rate of the reaction. The frequency factor, A, in

the equation is approximately constant. The validity of the Arrhenius equation can be tested by taking the (ln) of both sides of the equation.

$$\ln K = \ln A - \frac{E_a}{Rt} \tag{5b}$$

A plot of lnK Vs 1/T at 3 different points was plotted to evaluate Ea and A

Computer simulation via Time-series Analysis

Regression Equation - - - (6)

- i. Y_t = S_t * I_t
- ii. Deforming moving average {MAD [4]}
- iii. Deforming central moving average {CMA}
- iv. Deforming seasonal and irregular components = {Y_t/CMA * S_t I_t}
- v. Deforming only seasonal component {S_t}
- vi. Deseasonalized {Y_t/ S_t}
- vii. Deforming T_t = {I + slope *t} - Regression analysis to determine I and slope
- viii. Plot graph

3. RESULTS AND DISCUSSION

The variations in vitamin C concentration of the lettuce vegetable at room and cold temperatures are presented in Table 1. At room temperature range of 17.5 to 21 °C and cold temperature of 6.5 to 9.5 °C, the lettuce degrades within two (2) day intervals from 898.41 mg/100 g to 0.80, 0.7085, 0.4085 mg/100 g and 895 mg/100 g to 18.98, 16.89, and 12.76 mg/100 g on the 9th day respectively. As can be observed, the concentration of vitamin C decrease steadily as storage time and temperature increase in all the vegetable samples. Injury to the plant tissues affects both the rate and the extent of water loss, this is the reason why leafy vegetables such as lettuce lose water at a higher rate than potatoes and apples (27). This confirms the fact that vitamin C in fruits and vegetables degrade during processing and storage. Therefore, ascorbic acid is usually selected as the most frequently measured nutrient to evaluate nutrient loss during storage. With so many important roles, the retention of vitamin C in products is regarded as a reliable and representative index during processing (28). A visual inspection of the kinetic plots of models (Fig. 1) from integral law equation (1) and order of reaction n = 0, 1 and 2 ;(2a), (3a) (4a) at both temperature ranges shows that the first order model fitted the kinetic data best in all the temperatures concerned. This shows that the first-order model fits the kinetic data best in all the vegetable samples stored at room and cold temperatures.

Table 1: Vitamin C (mg) in lettuce at various room and cold temperatures.

Time (day)	17.5 °C	19.5 °C	21 °C	6.5 °C	7.5 °C	9.5 °C
1	898.41	898.41	898.41	895.75	895.75	895.75
3	578.22	541.22	490.45	578.47	470.25	440.68
5	65.28	57.81	47.94	331.30	297.89	256.93
7	26.84	7.95	5.75	61.78	48.87	38.78
9	0.80	0.7085	0.4085	18.98	16.89	12.76

This is confirmed by the goodness of fit data (Table 2), where the first order kinetics exhibited R^2 values; 0.922843, R^2 adjusted 0.897124, P-value; 0.009316, R^2 value; 0.966717, R^2 adjusted 0.955623 P-value; 0.002603, R^2 value; 0.967654, R^2 adjusted 0.956872, P-value; 0.002493 for lettuce stored at room temperatures of 17.5 °C, 19.5 °C and 21 °C, respectively. From Table 3, R^2 values; 0.940793, R^2 adjusted 0.921057, P-value; 0.006227, R^2 value; 0.948852, R^2 adjusted 0.931802, P-value; 0.004987, R^2 value; 0.955274, R^2 adjusted 0.940365, P-value; 0.00407, for lettuce stored at cold temperatures of 6.5 °C, 7.5 °C and 9.5 °C respectively. The R^2 values are the highest and P - values the lowest.

Thus, the vitamin C degradation kinetics in lettuce under various temperature ranges is best described by first-order kinetics. This implies that the rate of degradation at any time is dependent on the initial concentration of vitamin C in the salad vegetables. The model with maximum R^2 and minimum P-value is adjudged the best (28). However, according to Barbara et al. (30), it is possible to have a low R^2 for both linear and logistic regression and still have a model that is correctly specified in every respect. And vice versa, you can have a very high R^2 and yet have a model that is grossly inconsistent with the data.

At room temperature (Table 4), lettuce at 17.5 °C has the lowest rate constant of 0.855 day⁻¹ and the highest half-life of 0.8107 day compared with

at 19.5 °C and 21 °C with a rate constant of 0.925 day⁻¹, half-life of 0.7493 day and 0.991 day⁻¹, half-life of 0.6994 day respectively. On the other hand, a cold temperature at 6.5 °C has least rate constant of 0.497 day⁻¹ and the highest half-life of 1.3947 day compared with at 7.5 °C and 9.5 °C has rate constant of 0.51 day⁻¹, half-life of 1.3591 day and rate constant of 0.546 day⁻¹, half-life of 1.2695 day respectively. Again, comparing the least rate constants and highest half-lives (Table 4), it was deduced that at temperature of 6.5°C, the rate constant was 0.497 day⁻¹ and half-life of 1.3947day while at temperature 17.5 °C, the rate constant was 0.855 day⁻¹ and half-life of 0.8107 day. Invariably, the degradation of vitamin C at a temperature of 6.5 °C gave the lowest rate constant and highest half-life, which made it preferred over other temperatures.

Vitamin C belongs to the heat-sensitive substance. It is believed that the higher the storage temperature, the higher losses of vitamin C in the products (8,32,33). It is reported that drying temperature was the major factor controlling the degradation of vitamin C in lime residues and the higher drying temperature results in lower vitamin C content. The rate of deterioration is generally proportional to their respiration rate, which is often a good index to the storage potential of a crop. The higher the respiration rate, the shorter the shelf life and vice versa. Respiration rate can be used as a criterion to compare the perishability of fruits and vegetables (33).

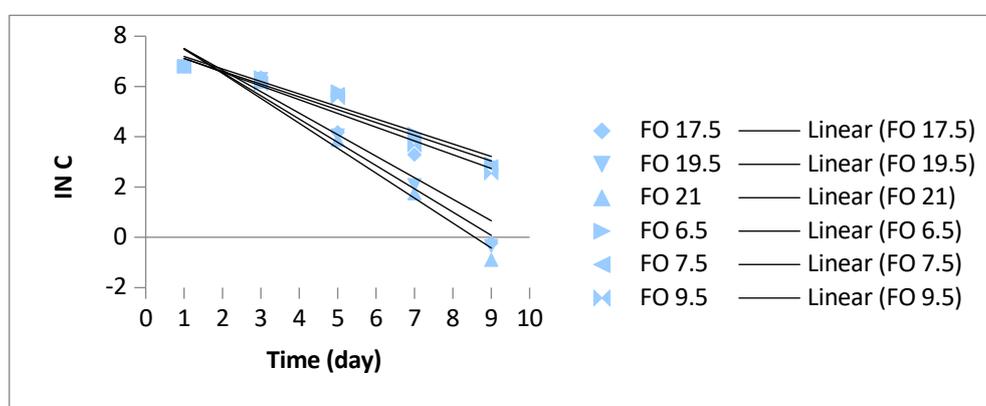


Figure 2: Plot of first-order kinetics for the lettuce at room and cold temperatures. FO: First-order, LN C: natural logarithm of the concentration of lettuce.

Table 2: Zero, first and second-order kinetic models at different room temperatures for lettuce.

KM ORDER	17.5 °C			19.5 °C			21 °C		
	R ²	R ² Adj	P- Value	R ²	R ² Adj	P- Value	R ²	R ² Adj	P- Value
ZO	0.842281	0.789708	0.02796	0.834523	0.779364	0.030128	0.820977	0.761303	0.034062
FO	0.922843	0.897124	0.009316	0.966717	0.955623	0.002603	0.967654	0.956872	0.002493
SO	0.524614	0.366152	0.1664	0.569261	0.425682	0.140523	0.555043	0.406725	0.148512

Table 3: Zero, first and second-order kinetic models at different cold temperatures.

KM ORDER	6.5 °C			7.5 °C			9.5 °C		
	R ²	R ² Adj	P- Value	R ²	R ² Adj	P- Value	R ²	R ² Adj	P- Value
ZO	0.955634	0.940846	0.00402	0.919219	0.892292	0.009991	0.89842	0.86456	0.014184
FO	0.940793	0.921057	0.006227	0.948852	0.931802	0.004987	0.955274	0.940365	0.00407
SO	0.71502	0.620027	0.071126	0.737742	0.650323	0.062248	0.729436	0.639248	0.065435

ZO – zero order, FO – first order, SO – second order, RT – Room Temperature, RT – room temperature, CT- Cold Temperature, KM – kinetic model.

Table 4: Rate constants, kinetic energies, and proposed models at RT and CT.

PT	T °C	k (day ⁻¹)	Half-life	Proposed Model	T (1/F)°C	E _A kcal/mol
RT	17.5	0.855	0.8107	ln(C) = ln(C ₀) - 0. 855t	0.015748031	10.2220
RT	19.5	0.925	0.7493	ln(C) = ln(C ₀) - 0.925t	0.01490313	
RT	21	0.991	0.6994	ln(C) = ln(C ₀) - 0.991t	0.014326648	
CT	6.5	0.497	1.3947	ln(C) = ln(C ₀) - 0.497t	0.022883295	30.4706
CT	7.5	0.51	1.3591	ln(C) = ln(C ₀) - 0.51t	0.021978022	
CT	9.5	0.546	1.2695	ln(C) = ln(C ₀) - 0.546t	0.020366599	

Table 5: First-order kinetics trendline equation and R squared value for lettuce at room and cold temperatures.

Lettuce at room and cold temperatures (°C)	Y intercept	R square
17.5	-0.8559x + 8.3606	0.9228
19.5	-0.9256x + 8.4038	0.9667
21	-0.9919x + 8.5034	0.9677
6.5	-0.4973x + 7.6919	0.9408
7.5	-0.5103x + 7.6242	0.9489
9.5	-0.5467x + 7.6611	0.9553

The degradation rate of vitamin C is less for all the samples at cold temperatures; in particular, the degradation rate is the lowest in lettuce with a rate constant of 0.497 day⁻¹ and a half-life of 1.3947 day. As shown in Table 4, storage is best done at a cold temperature of 6.5 °C, according to the established model. Lowering temperature during handling, transportation, and storage is the most effective means of extending the shelf life and reducing the loss of quality by lowering the metabolic processes such as respiration, transpiration, and ethylene production. However, vitamin C can be easily degraded because of its sensitive to various external factors, especially high temperature, oxygen, and light.

This indicates that the magnitude of the rate constant reflects the rate of reaction; the inference is that degradation of vitamin C occurred lower in lettuce vegetables stored at 6.5 °C under the same conditions. The half-life is longer for all analytical salad vegetable samples at cold temperatures implying that the rate of degradation of vitamin C is less as compared to storing at room temperature. Furthermore the kinetic models were developed based on the predicted initial contents, measured contents and storage time, from Table 4, the proposed models at 17.5 °C, 19.5 °C and 21 °C were: $\ln(C) = \ln(C_0) - 0.855t$, $\ln(C) = \ln(C_0) -$

$0.925t$, $\ln(C) = \ln(C_0) - 0.991t$ and at 6.5 °C, 7.5 °C and 9.5 °C were; $\ln(C) = \ln(C_0) - 0.497t$, $\ln(C) = \ln(C_0) - 0.51t$, $\ln(C) = \ln(C_0) - 0.546t$. The first order model kinetics forecasted on the 21st day show: -9.61288, -11.0329, -12.3264 for room temperatures and -2.75068, -3.09208, -3.81868 for cold temperatures respectively indicating forecast at 6.5°C the best, through mathematical model created on the computer and behavior of the model explored by running the simulation (forecast). The keeping quality of lettuce at a cold temperature of 6.5 °C is better than the rest. From Table 4 and Fig.4 and 5, lettuce room temperatures of 17.5 °C, 19.5 °C and 21 °C had activation energy of 10.2220 kcal/mol while lettuce cold temperatures of 6.5 °C, 7.5 °C and 9.5 °C had activation energy of 30.4706 kcal/mol. The activation energy for ascorbic acid degradation in lettuce concurs with the range reported by Mauri et al. (32). The activation energy represents the minimum energy required for a chemical reaction to occur. For vitamin C degradation in lettuce, the reaction involved the conversion of ascorbic acid (vitamin C) to dehydroascorbic acid (an inactive form). This process required a certain energy threshold to overcome the energy barrier, represented by the activation energy (Ea). Elevated temperatures reduce Ea, increasing the degradation rate (33).

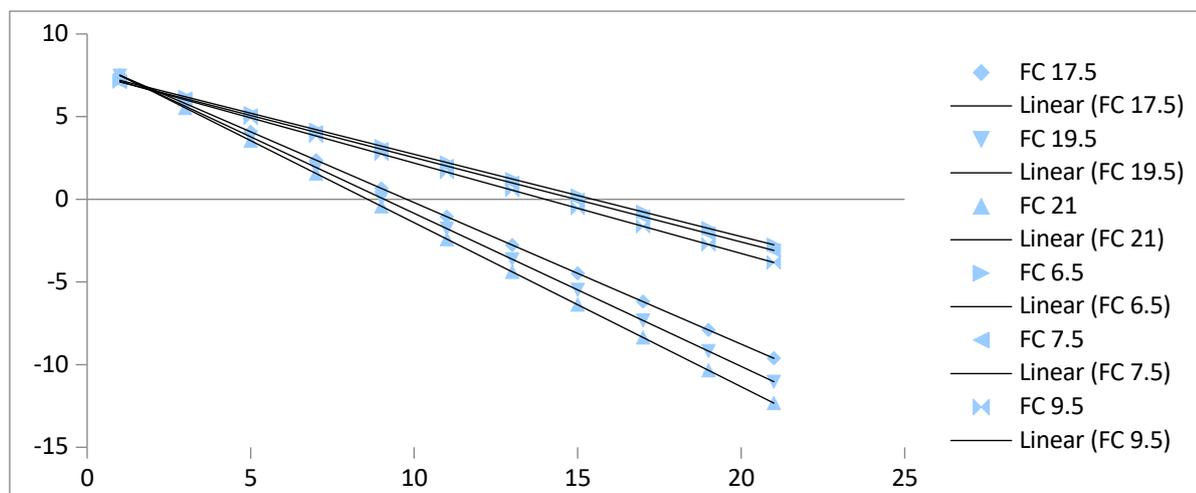


Figure 3: Computer simulation analysis (forecast) for lettuce at room and cold temperatures.

Table 6: First-order kinetics forecast Trendline equation and R Squared value for lettuce at room and cold temperatures.

Lettuce at room and cold temperatures (°C)	Y intercept	R square
17.5	-0.8559x + 8.3606	1.0
19.5	-0.9256x + 8.4038	1.0
21	-0.9919x + 8.5034	1.0
6.5	-0.4973x + 7.6919	1.0
7.5	-0.5103x + 7.6242	1.0
9.5	-0.5467x + 7.6611	1.0

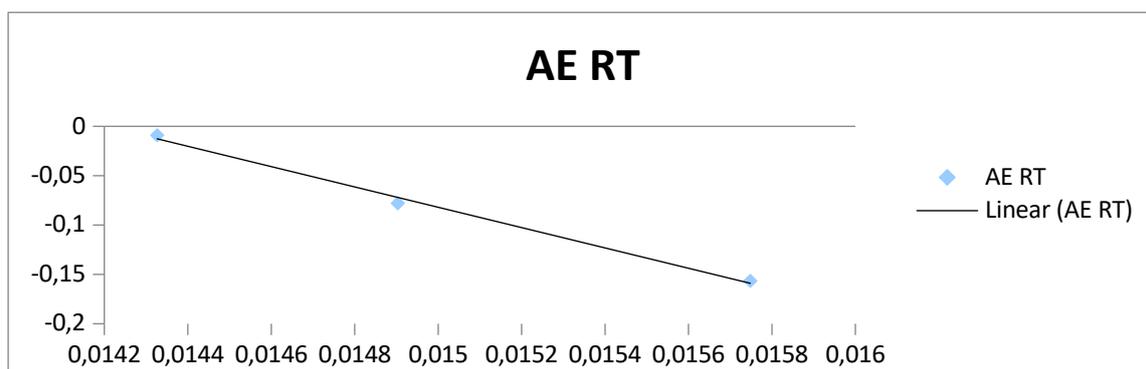


Figure 4: Arrhenius plot for lettuce at room temperature.

Table 7: Arrhenius trendline equation and R squared value for lettuce at room and cold temperatures.

Lettuce at room and cold temperatures (°C)	Y intercept	R square
RT	-103.06x + 1.4639	0.9951
CT	-37.942x + 0.1657	0.9912

RT: room temperature; CT: cold temperature

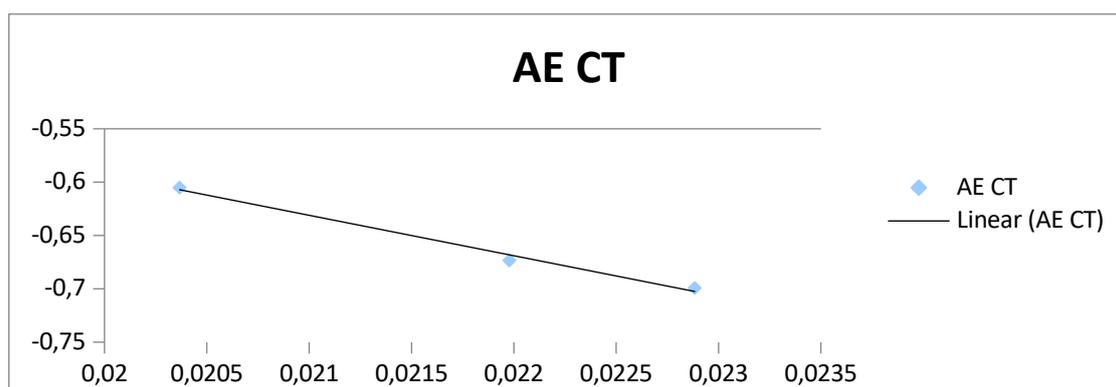


Figure 5: Arrhenius plot for the lettuce vegetable at cold temperature.

Among the temperatures and storage conditions studied, lettuce at cold temperatures has the highest activation energy, implying a lower degradation rate.

4. CONCLUSION

The rate of vitamin C degradation in the lettuce under room, cold temperatures and defined storage methods investigated in this study followed the first-order reaction kinetics. This indicated that the rate of degradation is dependent on the vitamin C concentration in the vegetable. There was a lower rate of vitamin C degradation at cold temperature storage of 6.5 °C compared to other temperatures. This implied that temperature is the major factor controlling the degradation of vitamin C. This is reflected in its lower rate constant, longer half-life, higher forecast, and activation energy values. This impresses the fact that the storage of lettuce at cold temperatures is preferable in terms of vitamin C retention.

5. CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

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