

Kinetic Modeling of Vitamin C Degradation in Commonly Consumed Salad Vegetables at Room Temperatures using Time Series Analysis (Forecast)

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ABSTRACT: Vitamin C (ascorbic acid) is one of the most important and popular vitamins, and is contained in most fruits and vegetables; the problem with vitamin C is its easy degradation during pre-treatment and storage. In this study, kinetics modeling of thermal degradation of ascorbic acid (Vitamin C) in lettuce, cabbage and carrot salad vegetables were investigated when dried at various room temperature ranges of (17.5 – 21)^oC. Samples after drying were ground to find dust and High Pressure liquid chromatographic (HPLC) was used for determination of the AA of the vegetable salad samples which consisted of an isocratic elution procedure with UV-Visible detection at 245nm. The rate constants and half-lives were calculated using the integrated law method. Activation energy and forecast were determined using Arrhenius equation and time series analysis. Degradation of ascorbic acid in the salad vegetables under the same pretreatment procedure followed the first-order kinetic model, as the average coefficient of determination (R^2 -value) was greater than 0.91. The rate constant of ascorbic acid degradation for Lettuce, Cabbage and carrot under the same room temperature condition of 17.5^oC were 0.7806, 0.1141 and 0.2634 day⁻¹ respectively. Their half-lives were 0.8879, 6.0749 and 2.6315 day⁻¹ respectively, mean values 1.3375, 5.9493, 3.2210 respectively, The activation energies; 0.48575, 4.8353, 1.4519 Kcal/mol respectively. $\ln C$ (Forecast) at day 9 lettuce (0.3056), at day 66 cabbage (0.0514), at day 25 carrot (0.3504). The proposed models were $\ln(C) = \ln(C_0) - 0.7806$, $\ln(C) = \ln(C_0) - 0.1141$, $\ln(C) = \ln(C_0) - 0.2634$. The most appropriate salad vegetable under the same room temperature drying and storage is the cabbage because its rate constant depicted from the model equations was lower, half life longer, mean value bigger, activation energy higher, forecast longer.

Keywords: HPLC, Lettuce, Cabbage, Room Temperature, Ascorbic Acid, Rate Constant, Activation Energy, Forecast

1. Introduction

As one of the most important phytochemicals, vitamin C (ascorbic acid, AA) is absolutely required in the human diet since humans lack gluconolactone oxidase enzyme and cannot synthesize vitamin C and entirely rely on dietary sources¹. The recommended dietary allowance (RDA) of vitamin C absorption are 75 mg/day, 90 mg/day, 45 mg/day for adult women, adult men, and children 9-13 years old, respectively². Vegetables and fruits are the major source of natural vitamin C and are present in reduced (L-ascorbic acid, AsA) and oxidized (L-dehydroascorbic acid monomer, DHA) form. Both AsA and DHA exhibit vitamin C activity and the AsA could transform into DHA by enzymatic and nonenzymatic oxidation during processing and storage^{3,4}. With so many important roles, the retention of vitamin C in products has been regarded as a reliable and representative index during their processing⁵. However, in aqueous solutions or in foods, its stability is related to the storage conditions and to the composition of the matrix⁶. The vitamin C has the least stability among all kinds of vitamins and is easily destroyed during processing and storage, depending on many variables such as pH^{7,4}, temperature^{8,9,10,11} and the presence of enzymes¹², oxygen, hydrogen peroxide¹³, and metallic catalyzers^{14,15,16}. Depending on these factors, two different types of degradation occur: aerobic and anaerobic degradation.

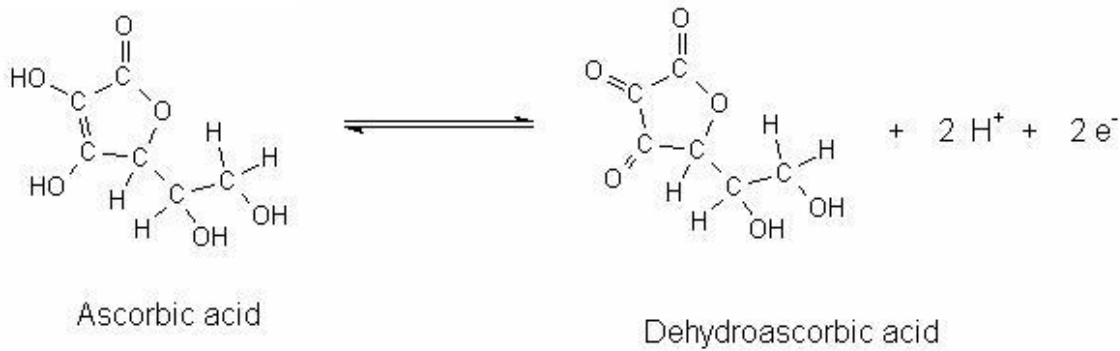


Fig 1.1 Oxidation of ascorbic acid to dehydroascorbic acid under aerobic conditions

The ascorbic acid is oxidized to dehydroascorbic acid under aerobic conditions, followed by hydrolysis and further oxidation¹⁷. The degradation mechanisms of vitamin C and the main influencing factors are shown in Fig 1.1 Vegetables generally refer to those plants which are consumed in relatively small quantities as a side dish or a relish with the staple food. They are the leaves, roots or stems of herbaceous plants. Nutritionally, they are good sources of vitamins, minerals and fibre. Vegetables also harbour microorganisms which are from the soil on which they are planted, the manure used to improve the quality of the soil or the water used for irrigation purposes. When consumed raw, they may endanger the health of the consumers especially when the pathogenic stains are involved. They are also rich sources of antioxidants such as vitamins A, C and E, carotenoids, polyphenolic components, and flavonoids which prevent the attack of free radicals thus reducing the risk of carcinogenic illnesses.¹⁸ Consumption of antioxidants in food via natural sources is good for the prevention of cardiovascular diseases, especially arteriosclerosis¹⁹. Vegetables are also low in fat and energy with high carbohydrate and fiber contents, providing significant levels of some micronutrients¹⁸.

Lettuce (*Lactuca sativa L*), a temperate annual or biennial plant is most often grown as a leafy vegetable. It is typically eaten cold and raw in salads, hamburgers and many other dishes. It is a widely grown and popularly consumed leafy vegetable because it contains vitamin C, polyphenols, and a 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)¹⁸.

Cabbage (*Brassica oleracea*) was picked wild and used predominantly as medicinal herb, prior to domestication. It is now a common vegetable used in salads and sauerkraut. Cabbage is one of the most highly rated leafy vegetables and marvelous food items. This vegetable is chiefly valuable for its high mineral, vitamin content and alkaline salts. The medicinal value of cabbage also includes the wonderful cleansing properties, their content of tartronic acid which inhibits fat formation, and the possession of elements and factors which enhance the immunity of the body and arrests ageing, cancer as well as possession of oestrogenic activities. Cabbage is an excellent source of Vitamin C¹⁸. The detoxifying effect of cabbage is due to the high content of vitamin C and sulfur in it.

Carrot (*Daucus carota*) is a root vegetable, usually orange, purple, red, white or yellow in color, with a crisp texture when fresh. It is a rich source of β -carotene and contains other vitamins, like thiamine, riboflavin, vitamin B-complex and minerals.²⁰ reported the consumption of carrot mainly as raw, juice, salads, cooked vegetable, sweet dishes etc. Carrot (*Daucus carota*) is one of the most versatile crops grown extensively in various countries. Carrot is valued as nutritive food mainly because, it is a rich source of β -carotene and contains appreciable amounts of thiamine and riboflavin. It has been reported that carrot has diuretic and nitrogen balancing properties as well as being effective in elimination of uric acid²⁰. Carrots are widely used in many cuisines, especially in the preparation of salads, and carrot salads are a tradition in many regional cuisines.

Drying under room temperature is one of the most commonly used technologies to prevent deterioration and prolong the shelf-life of fruits and vegetables. Temperature is the most important factor during drying and many researches explored the relationship between vitamin C retention and temperatures and the degradation kinetics. Vitamin C decreased as the product temperature increased and moisture content decreased. The degradation might be from both vitamin C oxidation and thermal destruction.²¹ studied ascorbic acid degradation kinetics in tomatoes at different drying conditions. It was reported that the degradation rates were dependent on samples treatment before drying, as well as on drying temperature. Increasing drying temperature led to higher degradation rates. Except drying temperature, the material thickness, relative humidity and drying time also influenced the ascorbic acid content. The effect of relative humidity on vitamin C retention is complex. As increasing relative humidity is a likely result to increasing drying time, which has negative on vitamin C retention. On the other hand, increasing relative humidity can cut the odds of oxidation reactions, which has positive effect for vitamin C retention. It should be noted that when drying processes are performed at very low

temperature or/and in modified atmosphere, the drying time is not a critical factor.²²The mathematical model as a tool has been widely applied to predict vitamin C content of various materials during drying²³.

Kinetics can be defined as the rate at which reaction occurs. Changes occur at certain reaction rates. Kinetic modeling enables to describe these changes and their rates quantitatively. Kinetic modeling also enables us understand the basic reaction mechanisms vital for quality modeling and control. The degradation kinetics of ascorbic acid in model systems conforms to first order kinetics, however, in food systems the kinetics is somehow complex²⁴. The complexity of the degradation mechanisms hinders the development of mechanistic models, and pseudo-kinetic model such as zero order, first-order or second-order kinetics are often applied in order to obtain a good fit to the experimental data. The model that gives the highest coefficient of determination value (R²) value is regarded as the best fit for the analysis²⁵. 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, Cabbage and carrot under the same room temperature conditions, so as to recommend the best; (ii) to develop kinetic models for predicting vitamin C degradation in lettuce, cabbage and Cabbage under the studied conditions. (iii) To predict the future values (forecast)

2. Methods and Materials

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 18MΩcm⁻¹ resistivity purified with a milli-Q system (Millipore, Bedford, USA) was used. Ascorbic acid stock standard solution was prepared in water and stored in a glass-stopper bottle at 4⁰C in the dark²⁶

2.2 Sample Preparation

Lettuce, Cabbage and Carrot were sourced, matured and fresh from fruits and vegetable market located in Yankaba, Nasarawa local Government of Kano state Nigeria which lies between Longitude 7⁰ 54' and 9⁰ 06' East and Latitude 11⁰ 37' and 12⁰ 21' North. The fresh and matured vegetable salad (900g) each were immediately washed with clean water to remove dirt and soil, and then drained with muslin cloth to remove unwanted water. Stems were removed by cutting with a sharp and washed knife to avoid contamination. The liquid extracts were used to assess the initial ascorbic acid degradation during room temperature storage. The remaining lots were spread evenly on labeled trays and dried under various room temperatures range of 17.5 – 21⁰C and relative humidity range of 50 – 61% using a thermometer and hygrometer. The initial sample served as the control. After drying they were blended, filtered and liquid extract used to determine the final rate of degradation of ascorbic acid. Experiments were carried out in 3 triplicates analysis and the average of measurement was reported.

2.4. Kinetic modeling

The degradation of vitamin C was modeled using the integrated rate law. Different models were developed using the integral method of analysis. The integral law equation stated below;

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

was used to develop three models based on concentration (for order of reaction n = 0, 1 and 2) and their associated half-lives (t_{1/2}).

Zero order model (n = 0):

$$C = C_0 - kt \quad (2a)$$

$$t_{\frac{1}{2}} = \frac{C_0}{2k} \quad (2b)$$

First order model (n = 1):

$$\ln(C kt) = \ln(C_0) - kt \quad (3a)$$

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

Second order model (n = 2):

3. Results

Table 1: Vitamin C in Salad Vegetable at Room Temperature (17.5⁰C) and Time Variations

P/T	Time(day)	Lettuce Vit.C(mg/100g)	Cabbage Vit.C(mg/100g)	Carrot (vit.C)mg/100g
Room Temp	1	898.41	960.30	480.14
17.5 ⁰ C	3	578.22	841.41	130.13
	5	65.28	419.56	101.51
	7	26.84	405.78	83.18
	9	0.80	387.77	57.04
	11	-1.60	311.70	20.51

Table 2: Vitamin C in Salad Vegetable at Room Temperature (19.5⁰C) at Time Variations

PM	Time (day)	Let Vit.C(mg/100g)	Cabb Vit.C (mg/100g)	Carr (vit.C) mg/100g
RT	1	898.41	960.30	470.14
19.5 ⁰ C	3	541.21	770.41	120.74
	5	57.81	400.56	90.31
	7	7.95	387.78	70.18
	9	0.7085	334.77	40.97
	11	-1.01	290.70	15.12

Table 3: Vitamin C in Salad Vegetable at Room Temperature (21.5⁰C) at Time Variations

P T	Time (day)	LettVit.C(mg/100g)	CabVit.C(mg/100g)	Carr (vit.C) mg/100g
RT	1	898.41	960.30	454.14
21 ⁰ C	3	490.45	670.41	110.74
	5	47.94	300.96	84.81
	7	5.75	297.79	63.56
	9	0.4085	244.77	35.45
	11	-0.8004	204.34	10.06

Table no 1- 3: Show two day time series interval analysis for salad vegetables dried for eleven days at the drying temperature range of 17.5 -21⁰C. The degradation of vitamin C followed the order : lettuce > carrot > cabbage implying that Cabbage degradation was less for all the drying room temperature range.

Let = lettuce, Cab = cabbage, car = carrot

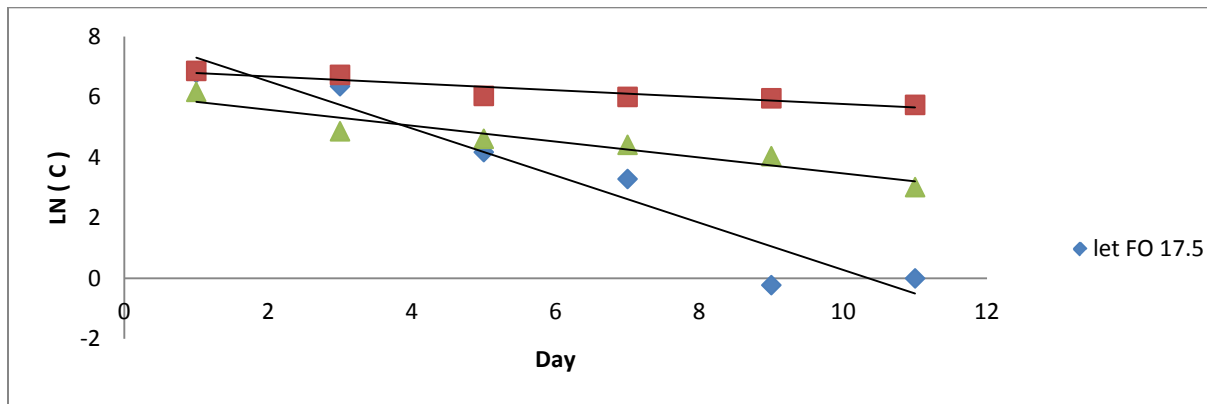


Fig.1: Plot of First Order Kinetics for the Blanched Vegetables at Room Temperature of 17.5°C

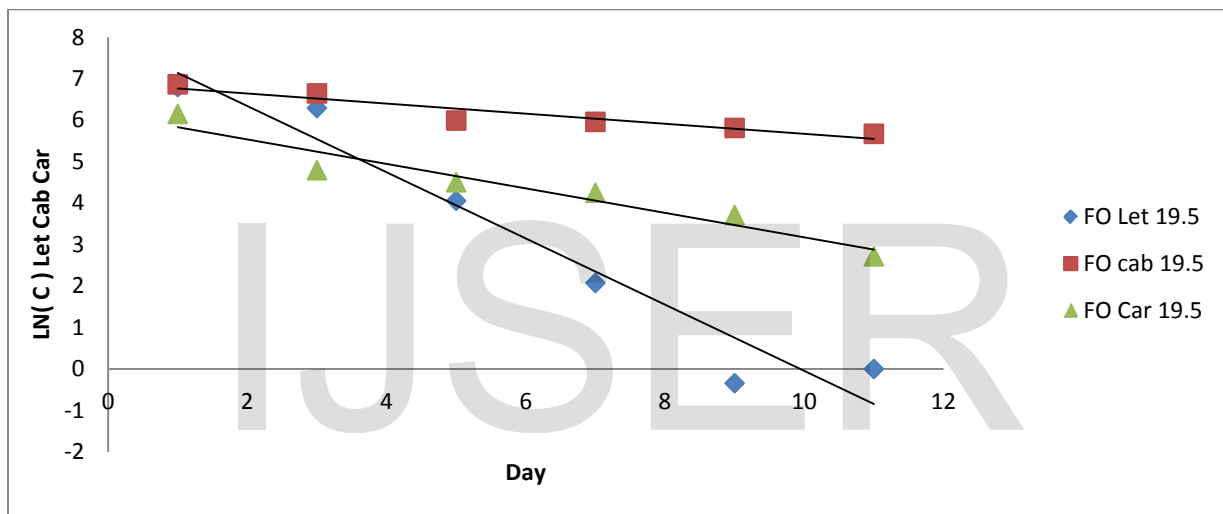


Fig.2: Plot of First Order Kinetics for the Salad Vegetables at Room Temperature of 19.5°C

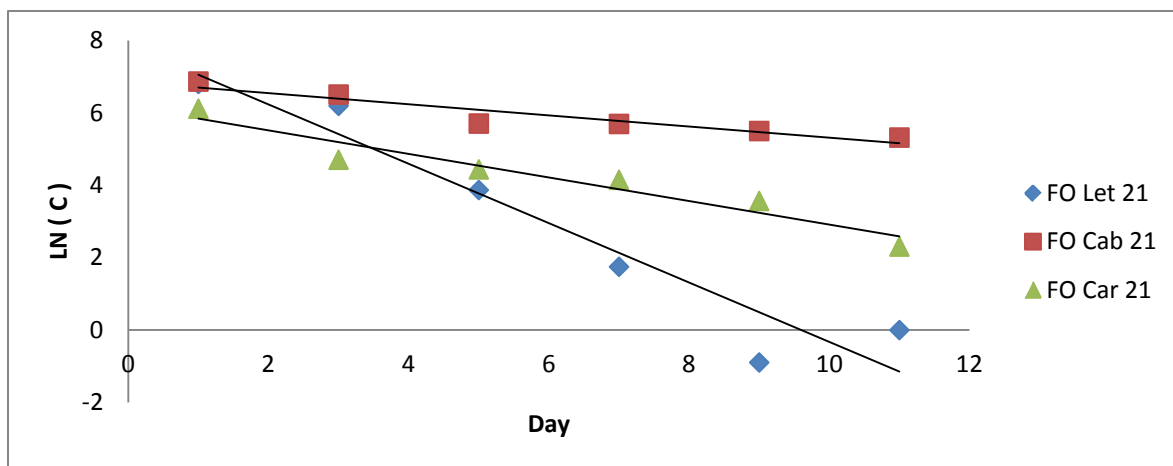


Fig.3: Plot of First Order Kinetics for the Blanched Vegetables at Room Temperature of 21°C

FO = First Order

Fig no 1- 3: Show plot of first order kinetics for the salad vegetables at room temperature range of 17.5 - 21°C at two day analysis interval for eleven days. The degradation of vitamin C followed the order: lettuce > carrot > cabbage implying that cabbage degradation was less for all the drying room temperature range.

Table no 4 below: show results of first order kinetic model regression analysis for salad vegetables at different room temperatures. The model that gives the highest coefficient of determination value (R² value) is regarded as the best fit for the analysis

Table 4: Results of Kinetic Model Regression Analysis for Salad vegetables at Different Room temperatures (First Order)

Veg	Treat	Temp (°C)	Statistics Parameters (First Order)		
			R ²	R ² adjusted	P- value
Lettuce	R T	17.5	0.9462	0.9193	0.027262
		19.9	0.9986	0.9980	0.0006649
		21	0.9981	0.9971	0.000948
Cabbage		17.5	0.7567	0.67561	0.055225
		19.9	0.8079	0.7439	0.03802
		21	0.8071	0.7429	0.03825
Carrot		17.5	0.8743	0.8324	0.0196671
		19.9	0.9136	0.8848	0.01106
		21	0.8857	0.8476	0.016987024

Table 5: Rate Constant kinetic Model Regression Analysis for Salad Vegetables at Different Room Temperatures

Vegetable	Temp(°C)	K (min ⁻¹)	Half-Life	Mean Value	AE Kcal/mol	Proposed Model
Lettuce	17.5	0.7806	0.8879	1.3375	0.4857549	ln(C) = ln(C ₀) - 0.7806
	19.5	0.7986	0.8679	0.1107		ln(C) = ln(C ₀) - 0.7986
	21	0.9919	0.6988	1.2259		ln(C) = ln(C ₀) - 0.9919
Cabbage	17.5	0.1141	6.0749	5.9493	4.835314	ln(C) = ln(C ₀) - 0.1141
	19.5	0.1215	5.7049	5.9867		ln(C) = ln(C ₀) - 0.1215
	21	0.1539	4.5038	5.7093		ln(C) = ln(C ₀) - 0.1539
Carrot	17.5	0.2634	2.6315	4.2210	1.451917	ln(C) = ln(C ₀) - 0.2634
	19.5	0.2954	2.3464	0.9285		ln(C) = ln(C ₀) - 0.2954
	21	0.3251	2.1321	3.7543		ln(C) = ln(C ₀) - 0.3251

Table no 5: shows result of first order rate constant, half – life, mean value, activation energy for salad vegetables at different room temperatures, which followed the degradation order: lettuce > carrot > cabbage

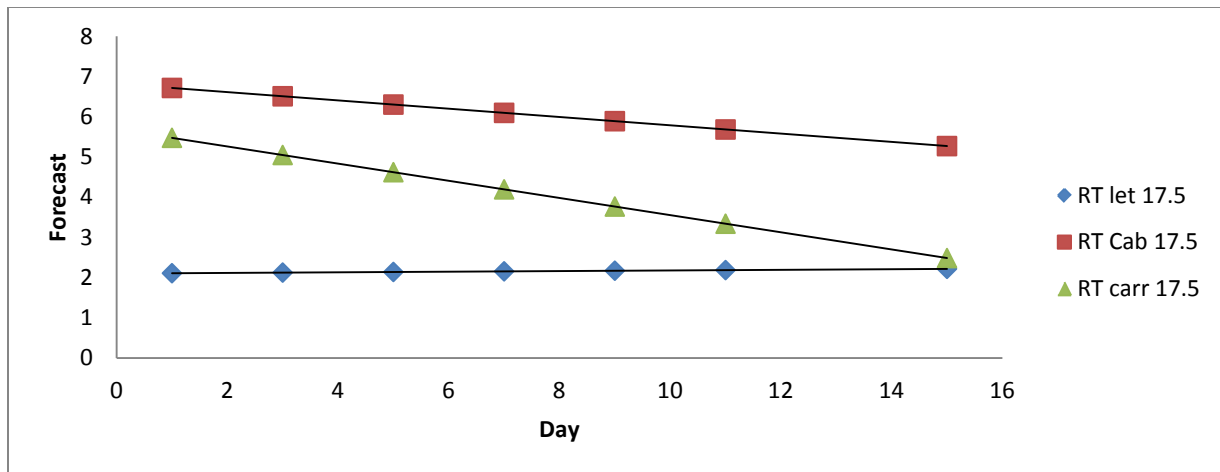


Fig.4: Time Series Forecast Analysis for Salad Vegetables at Room Temperature of 17.5⁰C under Kinetic Orders

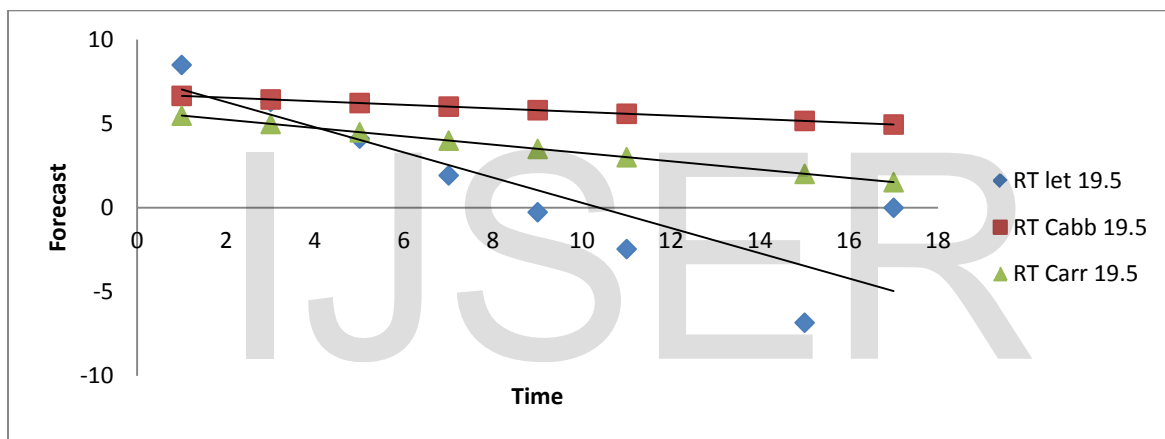


Fig. 5: Time Series Forecast Analysis for Salad Vegetables at Room Temperature of 19.5⁰C

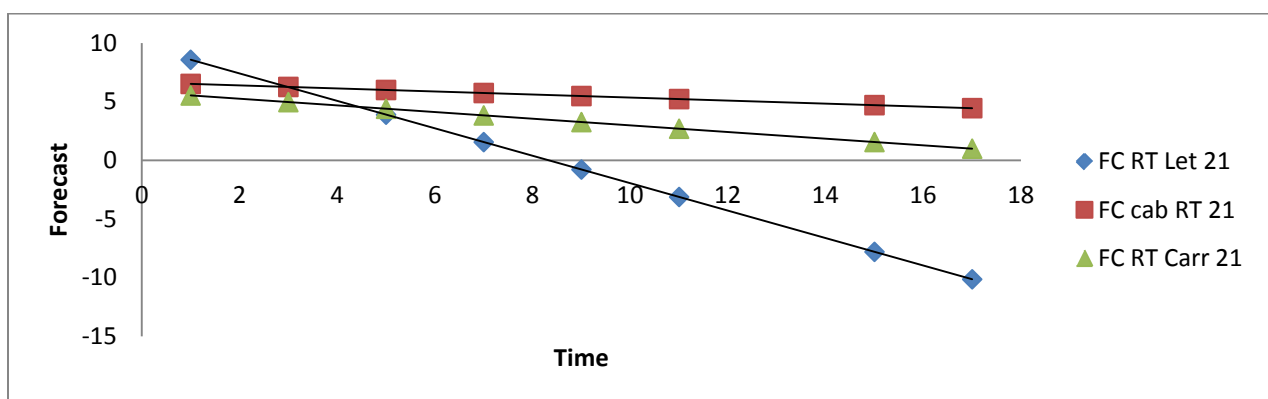


Figure.6: Time Series Forecast Analysis for Salad Vegetables at Room Temperature of 21⁰C

Fig no 4- 6: Show plot of time series forecast analysis for salad vegetables at room temperature range of 17.5 - 21°C. The degradation of vitamin C followed the order: lettuce > carrot > cabbage implying that cabbage degradation was less for all the drying room temperature range.

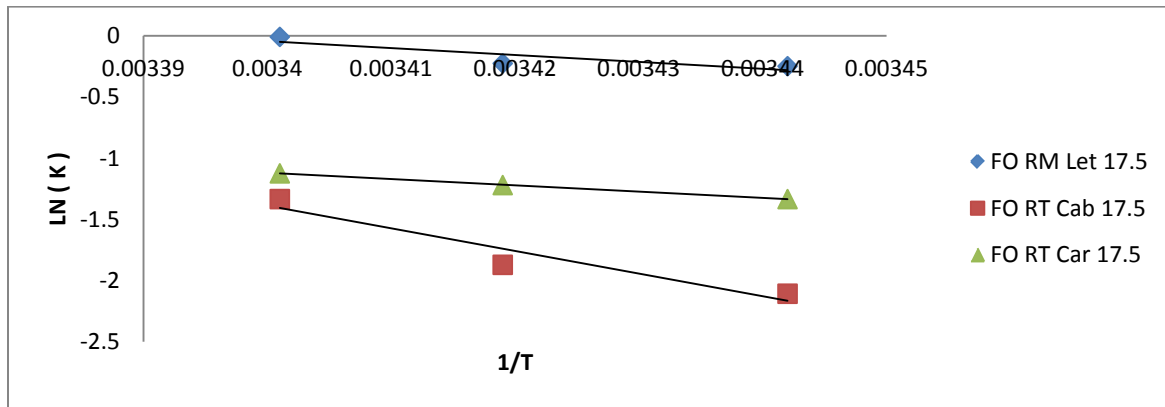


Fig.7: A Plot of Activation Energy of Salad Vegetables at Room Temperature

Fig no 7: Show plot of activation energy of salad vegetables at room temperature range of 17.5 - 21°C. The activation energy value of the salad vegetables followed the order: cabbage > carrot > lettuce.

4. Discussion

The variations in vitamin C concentration of the salad vegetables at room temperatures are presented in Table no 1 –3. At room temperature range of 17.5°C to 21°C, the lettuce, cabbage and carrot degraded within two(2) day intervals from 898.41mg/100g, 960.30mg/100g and 480.14mg/100g to -1.60mg/100g, 311mg/100g and 20.51mg/100g (17.5°C), 898.41mg/100g, 960.30mg/100g and 480.14 mg/100g to -1.01mg/100g, 290.70mg/100g and 15.12 mg/100g(19.5°C), 898.41mg/100g, 960.30mg/100g and 454.14mg/100g to -0.8004mg/100g, 204.70mg/100g and 10.06mg/100g for eleven (11) days respectively. As can be observed, the concentration of vitamin C decreased steadily as storage time and temperature increases in all the vegetable samples. Injury to the plant tissues affect both the rate and the extent of water loss, this is the reason why leafy vegetables such as lettuce lose water at higher rate than potatoes and apples²⁷. This confirms the fact that vitamin C in fruits and vegetables degrade during processing and storage. Besides, in a recent investigation by²⁸, the ascorbic acid content in a given mass of cabbage was found to be greater than that in an equal mass of lettuce. This is confirmed by this study; as the concentration of vitamin C in cabbage is greater than that in lettuce and carrot for the same mass of sample. Genetic makeup has a profound effect on the selection of a raw material for a given processing application. Cultivar and rootstock selection influence the composition, quality, storage potential, and response to processing characteristics that may be inherited²⁹. Therefore, ascorbic acid is usually selected as the most frequently measured nutrient to evaluate the nutrients loss during storage. With so many important roles, the retention of vitamin C in products has been regarded as a reliable and representative index during their processing^{5, 30}.

A visual inspection of the kinetic plots of models (Fig. 1 – 3) for lettuce cabbage and carrot at room temperature shows that the first order model fitted the kinetic data best in all vegetable samples. These R² values were the highest and P- values were lowest. It shows that the first order model fitted the kinetic data best in all the salad vegetable samples stored at room temperature. This is confirmed by the goodness of fit data (Table 4), where the first order kinetics exhibited R² values; 0.9462, P-value; 0.027263, R² value; 0.9986, P-value; 0.0006649, R² values; 0.7567, P-value; 0.055225 for Lettuce stored at 17.5°C, 19.5°C and 21°C respectively. R² value; 0.8079, P-value; 0.03802, R² value; 0.8071, P-value; 0.03825, R² values; 0.8743, P-value; 0.0196671 for cabbage at 17.5°C, 19.5°C and 21°C respectively. R² value; 0.9136, P-value; 0.01106, R² value; 0.9136, P-value; 0.01106, R² value; 0.8857, P-value; 0.016987024 for carrot at 17.5°C, 19.5°C and 21°C respectively. These R² values were the highest and P - values the lowest. Thus, the vitamin C degradation kinetics in lettuce cabbage and carrot is best described by a 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 R² and minimum P-value is adjudged the best³¹, though³² said for both linear and logistic regression, it is possible to have a low R² and still have a model that is correctly specified in every respect. And vice versa, you can have a very high R² and yet have a model that is grossly inconsistent with the data. At 17.5°C, from Table no 5, lettuce, cabbage and carrot at 17.5°C had least rate constants of 0.7806 day⁻¹, 0.1141 day⁻¹, 0.2634 day⁻¹ respectively. Vitamin C belongs to the heat sensitive substance. It is believed that the higher storage temperature the higher losses of vitamin C in the products^{12,33,34,6} 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. Also respiration is an indicator of metabolic activity of all living produce and plays a significant role in the postharvest physiology and deterioration of quality of plant foods. The rate of deterioration is generally proportional to their respiration rate, which is often a good index to the storage potential of a crop. Higher the respiration rate, shorter the shelf life and vice versa. Respiration rate can be used as a criterion to compare perishability of fruits and vegetables.³⁶ The rate of degradation of vitamin C is less for all the samples at 17.5°C, in particular, the rate of degradation was least in cabbage with rate constant of 0.1141 day⁻¹. Deduced from Table 5 is that storage is best done at room temperature of 17.5°C with the established model.²⁹ 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 and transpiration and ethylene production. However, vitamin C can be easily degraded and very sensitive to various external factors, especially high temperature, oxygen and light.⁶ This indicates that magnitude of the rate constant is a reflection of the rate of reaction; the inference is that degradation of vitamin C occurred lower in salad vegetable samples stored at 17.5°C under same principle of storage conditions. This trend manifested in the half-life of the samples which gives further credence to this fact.

The half-lives of the salad vegetables at room temperature from Table 5 exhibited 0.8879 min, 0.8679 min and 0.6988 min for lettuce, 6.0749 min, 5.7049 min and 4.5038 min for cabbage, 2.6315, 2.3464, 2.3464 for carrot at 17.5°C, 19.5°C and 21°C respectively. The half-life is longer for all analytical salad vegetable samples at 17.5°C implying that rate of degradation of vitamin C is less as compared to storing at 19.5°C and 21°C. In particular, cabbage stored at 17.5°C had longest half-life of 6.0749 min. The time at which the concentration of vitamin C in the samples reduces to half of its original amount (half-life) was longer in cabbage in lettuce and carrot salad vegetable samples.

Furthermore the kinetic models were developed based on the predicted initial contents, measured contents and storage time, from Table 5, the proposed models at 17.5°C, 19.5°C and 21°C respectively were: $\ln(C) = \ln(C_0) - 0.7806t$, $\ln(C) = \ln(C_0) - 0.7986t$, $\ln(C) = \ln(C_0) - 0.9919t$ for lettuce, $\ln(C) = \ln(C_0) - 0.1141t$, $\ln(C) = \ln(C_0) - 0.1215t$, $\ln(C) = \ln(C_0) - 0.1539t$ for cabbage, $\ln(C) = \ln(C_0) - 0.2634t$, $\ln(C) = \ln(C_0) - 0.2954t$, $\ln(C) = \ln(C_0) - 0.3251t$ for carrot.

From Table 1, 2 and 3 at 17.5°C, 19.5°C and 21°C storage, lettuce is already void of vitamin C at day 11, -1.60, -1.01, and -0.8004 mg/100g, cabbage still had 311.70, 290.70, 204.34, while carrot had 20.51, 15.12 and 10.06 but forecasting beyond experimental shown forecast (Ln C) at day 66, cabbage 0.0514, at day 25 carrot 0.3504, day 9 lettuce 0.3056. These imply that after day 66, 25 and 9 nothing else will be found in cabbage, carrot and lettuce respectively. From above the storage and keeping quality of cabbage at room temperature is better than the rest.

From Table 5 and Fig. 6, lettuce, cabbage and carrot at room temperature of 17.5°C, 19.5°C and 21°C had activation energies of 0.485755, 4.835314 and 1.451917 Kcal/mol respectively. Among the salad vegetables studied, cabbage had the highest activation energy, implying lower rate of degradation.

5. Conclusion

The rate of vitamin C degradation in the Lettuce, cabbage and carrot salad vegetables samples under the same defined storage method investigated in this study followed the first order reaction kinetics. This indicates that the rate of degradation is dependent on the concentration of the vitamin C present in the vegetables. There was lower rate of vitamin C degradation at room temperature storage of 17.5°C for all the salad vegetables than at 19.5°C and 21°C in particular for cabbage. Implying that drying temperature is the major factor controlling the degradation of vitamin C. These were reflected in their lower rate constant, longer half-life, higher forecast and activation energy values and in particular cabbage. This impresses the fact that storage of Cabbage at room temperature is more preferable in terms of vitamin C retention than Lettuce and carrot.

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