Kinetic Modeling of Vitamin C (Ascorbic Acid) Degradation in Blanched Commonly Consumed Salad Vegetables Using Computer Simulation Analysis

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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 processing. In this study, the degradation kinetics of vitamin C was determined in Lettuce and Cabbage, and the processing treatment considered was blanching at 70^0C of water differently for 5, 10, 15, 20,25,30,35 and 40 minutes. Samples were dried in mild temperature (15–20^0C) and ground to find dust and High Pressure liquid chromatographic (HPLC) was used for determination of the AA of vegetable salad samples which consisted of an isocratic elution procedure with UV-Visible detection at 245nm. The rate constants were calculated for both vegetable salad samples under the same processing method using the integrated law method; half-life was also calculated. Degradation of ascorbic acid in Lettuce and Cabbage under the same pretreatment procedure followed the first-order kinetic model, as the coefficient of determination (R^2-value) were 0.8981 and 0.9785 respectively. The rate constant of ascorbic acid degradation for Lettuce and Cabbage under the same blanching conditions were 0.099 min-1 and 0.088 min-1 respectively. The half-life of Lettuce and Cabbage were 420.0892 and 472.6004 seconds respectively. The most appropriate vegetable salad under the blanching pretreatment procedure is the cabbage because its rate constant depicted from the model equations was lower, and the half life longer, hence, slower rate of degradation. The first order forecast (Ln(C)), was 0.657964 for Lettuce and 1.330017 for Cabbage, which further authenticate that blanched Lettuce degradation was higher than Cabbage under the same pretreatment conditions.*
Keywords*: HPLC, Lettuce, Cabbage, Blanching, Ascorbic Acid, Rate Constant*

I. Introduction

Vegetables are low in fat and energy with high carbohydrate and fibre contents, providing significant levels of some micronutrients [1]. Cultivated Lettuce *(Lactuca sativa L.)* 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) [2].The major traditional use of lettuce is as a sleep inducer. Research into the extracts of Lettuce resulted in the isolation of a depressant chemical.This chemical, when admistered in experimental animals, showed significant sedative effects [3]. On the other side Cabbage or headed cabbage (comprising several cultivars of *Brassica oleracea*) is a leafy green or purple biennial plant, grown as an annual vegetable crop for its dense-leaved heads. It is an excellent source of vitamin C and vitamin K, containing more than 20% of the Daily Value (DV) for each of these nutrients per serving [4]. The Ancient Greeks recommended consuming the vegetable as a laxative and used cabbage juice as an antidote for mushroom poisoning [5, 6]. For eye salves, and for liniments used to help bruises heal [7].

Vitamin C (ascorbic acid, AA) is an important component of our nutrition. It prevents scurvy and protects the body from oxidative stress which might lead to cancer, inflammation, cardiovascular diseases, asthma and arteriosclerosis [8]. Plants can make it themselves as can some animals, but humans do not have the right enzyme (gulonolactone oxidase) to synthesize this vitamin, hence, ascorbic acid has to be supplemented mainly through fruits and vegetables. Ascorbic acid is known to be a vitamin sensitive to a number of factors, including pH, moisture content, light, oxygen and temperature [9]. Its degradation proceeds in both aerobic and anaerobic pathways and depends upon many factor, such as oxygen, light, heat, storage temperature and storage time [10, 11]. Oxidation of ascorbic acid occurs mainly during the processing of juices, whereas, anaerobic degradation mainly appears during storage [12]. The nutrient quality of food during processing has become increasingly important. The degradation of some nutrients such as vitamin C during processing might be a critical factor for the shelf life of some products such as juice concentrate, since vitamin C content of fruits undergoes destruction during storage [13]. Pretreatment are common in most of the drying process order to improve product quality,

storage stability and processing efficiency. In recent years, an improvement in quality retention of the dried products by altering processing strategy and pretreatment has gained much attention.

Blanching is a process of preheating the product by immersion in water or steam. The main purpose of blanching is to inactivate the naturally occurring enzymes present in foods, since enzymes are responsible for off-flavour development, discoloration or browning, deterioration of nutritional quality and textural changes in food materials. Other advantages are that it removes air-bubbles from vegetable surfaces and from intercellular spaces, reducing the initial microbial load, cleans raw food material initially, facilitating preliminary operations such as peeling, dicing, improving color, texture and flavor under optimum conditions [14,15,16]. Blanching may have disadvantages for example, it may change the texture, color and flavor because of the heating process [17, 18]. It increases the loss of soluble solids, such as vitamins, especially in the case of water blanching [19, 20].It changes the chemical and physical states of the nutrients and vitamins [21, 14, 22]. It has adverse environmental impacts, such as large water and energy usage and problem of effluent disposal. Time and temperature of blanching are the important factor for achieving optimum quality of the dried products [10]. The normal blanching temperature varies from 80^0 C to 100^0 C. Recently, low temperature and long-time blanching has been proposed for better texture and retention of some nutritional components [23, 24]. The temperature used in between 50^0 C and 70^0 C, blanching times correlated with the flavor and sensory attributes of dried fruits and vegetables [18]. Limiting blanching time, rapid cooling and alternative blanching methods and combinations may result in dried products with better flavor. Processing methods that include steam and microware blanching and non thermal enzyme inactivation such as high pressure and ohmic heating may hold out potential for future blanching processes with less detrimental effects or flavor while maintaining optimum texture. Therefore, a deep knowledge of vitamin C degradation such as the kinetic order, and rate constants are the basic requirements to define the shell life of fruit juice and related products [25]. Numerous analytical techniques are available for the determination of the vitamin C content in various fruits and vegetables, amongst them are HPLC, Iodometric titration, chromatographic methods, enzymatic methods, and electrochemical methods [26, 27, 28, 29]. 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 complex [30]. 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 (R^2) value) is regarded as the best fit for the analysis [31].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 and Cabbage under the same pretreatment conditions of blanching at 70^0C of water, so as to recommend the best; (ii) to develop kinetic models for predicting vitamin C degradation in Lettuce and Cabbage under the studied conditions. (iii) to predict the future values(forecast)

II.   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 (Darmastdt, 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 4^0C in the dark [32].

2.2 Sample preparation
Lettuce and Cabbage were sourced matured and fresh from fruits and vegetable market located in Yankaba, Nasarawa local Government of Kano state Nigeria which lies between Longitude 7^0 54' and 9^0 06' East and Latitude 11^0 37' and 12^0 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 leaves (100g) each was then blanched in two (2Litres) of water differently at 70^0C for 5, 10, 15, 20,25,30,35 and 40 minutes. The blanched samples were cooled immediately to stop further cooking under running tap water for 2minutes, drained differently using muslin cloth for 5minutes and the initial samples were blended in a Kenwood blender (Philips, HR 1702, Borehamwood, England, UK) and filtered with cheese-cloth. The liquid extracts were used to assess the initial of ascorbic acid degradation during blanching. The remaining lots were spread evenly on trays and dried under room mild temperature and relative humidity using hygrometer.

Untreated 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 replications and the average of measurement was reported.

2.3 Instrumentation

The HPLC system consists of Waters liquid chromatograph (Milford, MA, USA) equipped with a 600E multisolvent delivery system, an in-line degasser, a manual injection with 20 μL loop (Rheodyne 7125), and Waters 2487ʎ 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 the method done by [33]. About 100g of salad vegetable samples in each maturity stage were separately weighed and dried under mild temperature ($15–20^0$ C) and ground to find powder dust before extraction. Then obtained powder were weighed (1.0 g for each sample and subsequently extracted with 25mL of extractant solution, containing 5% MPA, at 10^0 C and in the dark. Extraction process was performed using a shaker for 4 h. All extractions were carried out in triplicate and obtained solutions were then filtered and stored at 4^0C before analysis. The injection of the extracts into HPLC system was performed twice

2.3.3. HPLC analysis

The liquid chromatographic method used for the determination of AA consisted of an isocratic elution procedure with UV-Visible detection at 245 nm. Separations were carried out on a 5 μm RP C18 column of 250mm × 4.6mm (Spherical, Optimals ODS-H, Capital HPLC, UK) fitted with a 5 μm RP C18 guard column of 20mm × 4.6mm (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).Flow rate of the mobile phase was 1.2mL min^{-1} and an injection volume of 20 μL was used in quantitative analysis. The temperature of analytical column was kept constant at 25^0 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 0.5–200mg L^{-1} and based on a 10-point calibration.

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;

$dC/dt = -K[C]^n$ ………………………………………………… …………………… (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):

$C = C_0 - Kt$ ………………………………………… ………………………… (2a)

$(t_{1/2}) = C_0/2K$ ……………………………………… …………………… (2b)

First order model (n = 1):

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

$(t_{1/2}) = \ln(2)/k$ ……………………………………………………… (3b)

Second order model (n = 2):

$1/C = 1/C_0 + Kt$ ……………………………………………………… (4a)

$t_{1/2} = 1/kC_0$ ……………………………………………… (4b)

Where, K = rate constant

C_0 = initial concentration of vitamin C in sample

C = concentration of vitamin C in sample at time t

$t_{1/2}$ = half-life of vitamin C in sample

Concentration or a function of concentration was plotted against time for each model and regression analysis was used to determine the 'Goodness of fit' employing Minitab 17.3.1, 2016 [34]. Goodness of fit is

characterized by coefficient of determination (R^2), R^2 adjusted is the percentage of response variable variation that is explained by its relationship with one or more predictor variables, adjusted for the number of predictors in the model. Regression p- Value when small (typically ≤ 0.05) indicates strong evidence against the null hypothesis. The kinetic model could be developed based on the predicted initial contents, measured contents and processing time. This was achieved using the statistical program [35].A multiple comparison of the treatment means was performed by Duncan's New Multiple Range test. The model with maximum R^2 and minimum p- Value is adjudged the best [36, 37].

III. Results And Discussion

The variations in vitamin C concentration of vegetables samples during processing is presented in tables 1 and 2.Table 3 summarize the results of kinetic model regression analysis, while Table 4 compares vitamin C first order kinetics degradation parameters for the samples under the same processing method with Zero and Second order kinetics. As can be observed in Table 1 and 2 the concentration of vitamin C decreased steadily with time during processing in all the samples. This confirms the fact that vitamin C in salad vegetables degrade during processing [31]. This is in an agreement with the report of earlier workers on blanching of fruits and vegetables   that ascorbic acid is water soluble that makes it prone for leaching from cells [38]. It is thermally labile, pH-, metal- ion-, and light-sensitive, and can be degraded by ascorbic acid oxidase [38, 39] and in accordance with the degradation kinetics of ascorbic acid in model systems as put forth by Liao [30]. Therefore, ascorbic acid is usually selected as the most frequently measured nutrient to evaluate the nutrients loss during blanching process. The preservation of

ascorbic acid after blanching is a good indicator for the preservation of other nutrients [40, 41].The vitamin C concentration of the salad vegetable samples with respect to time decreased during processing, but in different degrees, depending on nature of salad vegetables. This is in agreement with the report of earlier workers on citrus and strawberry fruit juices [13, 25], and in accordance with the degradation kinetics of ascorbic acid in model systems as put forth by Liao and Seib [30]. Besides, in a recent investigation by NEWHEALTHADVISOR, 2017 the ascorbic acid content in a given mass of Cabbage was found to be greater than that in an equal mass of Lettuce [34]. This is confirmed by this study; as the concentration of vitamin C in Cabbage is greater than that in Lettuce for the same mass of sample after blanching. A visual inspection of the kinetic plots of models (2a),(3a) and (4a) presented in Figures 1-3 for Lettuce, and in Figures 4-6 for Cabbage, shows that the first order model fitted the kinetic data best in all vegetable samples.

Table 1. Vitamin C concentration in Lettuce during processing

Processing Treatment	Sample Code	Minutes	Vitamin C(mg/100g)
Blanching	LE1	5	29.36
	LE22	10	61.93
	LE43	15	30.36
	Le51	20	15.19
	Le52	25	10.06
	Le53	30	7.1
	Le54	35	2.98
	Le55	40	1.27

Table 2. Vitamin C concentration in Cabbage during processing

Processing treatment	Code	Time(minutes)	Vitamin C(mg/100g)
Blanchining	CA8	5	68.72
	CA29	10	59.57
	CA50	15	36.49
	Ca1	20	25.48
	Ca11	25	15.41
	Ca22	30	6.96
	Ca33	35	5.32
	Ca37	40	4.3

Table 3. Results of kinetic model statistical analysis for Lettuce and cabbage

Processing method	Vegetables Blanched	Kinetic Order(n)	R^2	Adjusted R^2	P-Value
Blanched at70^{0}C H$_2$0	Lettuce	0	0.662439	0.606179	0.013947
	Cabbage	0	0.909433	0.894338	0.00024
	Lettuce	1	0.898063	0.881073	0.000345
	Cabbage	1	0.97848	0.974893	0.00000314
	Lettuce	2	0.643376	0.583938	0.016613
	Cabbage	2	0.887244	0.868451	0.000468

Table 4. Comparison of first order kinetic parameters, proposed model and Paired Samples Statistics

Processing Method	Vegetables Blanched	Kinetic Order(n)	Mean	Rate Constant K (Min⁻)	Half Life $t_{1/2}$	Forecast ln(C)	Proposed Model
	Lettuce	1	2.4049	0.099	420.0892sec	0.657964	$\ln(C) = \ln(C_0) - 0.099t$
	Cabbage	1	2.8697	0.088	472.6004 sec	1.330017	$\ln(C) = \ln(C_0) - 0.088t$

This is confirmed by the goodness of fit data in Table 3.The first order kinetics exhibited R^2 values; 0.8981, P-value; 0.000345 for Lettuce and R^2 values; 0.97848, P-value; 0.00000314 for Cabbage under the same blanching conditions. The R^2 value of Cabbage was highest with P- value lowest as compared with Lettuce,R^2 lowest with P-value highest.Signifying that as the R^2 increases, the P-value decreases. Thus, the vitamin C degradation kinetics in Cabbage and Lettuce can be 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. Furthermore, the rate constant of Salad vegetable (Cabbage) was lower than that of Lettuce.
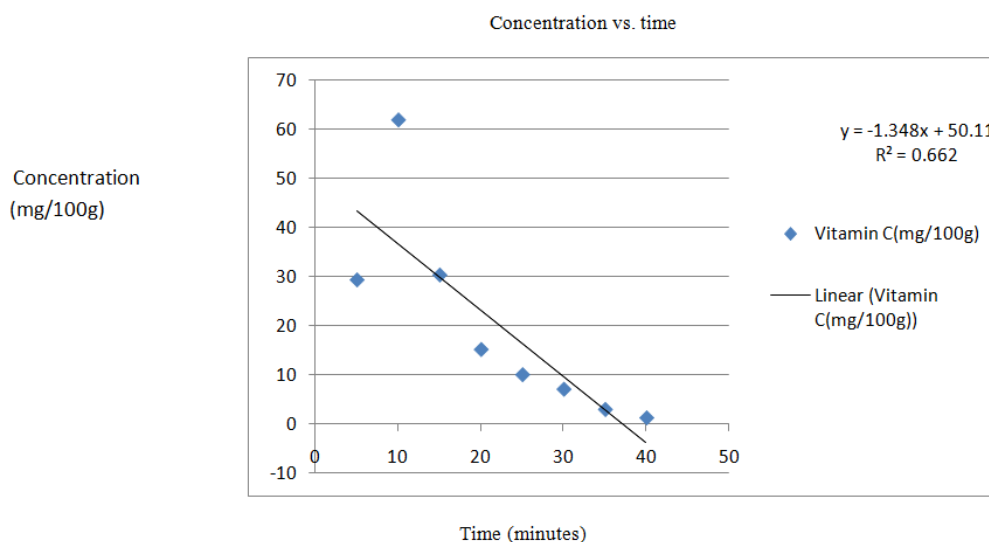

Fig. 1. A plot of zero order kinetics for Lettuce (concentration vs. time)
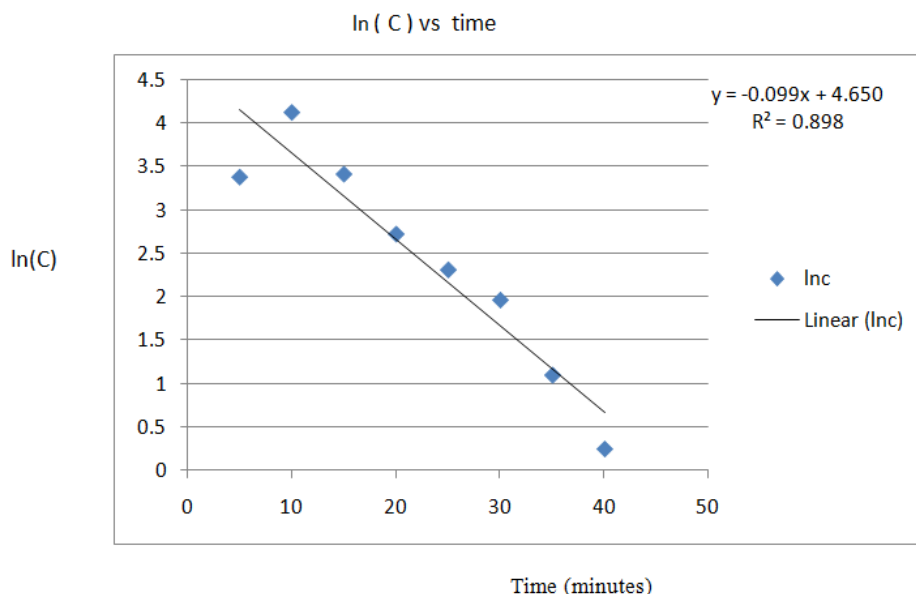

Fig. 2. A plot of first order kinetics for Lettuce ln(C) vs. time) C = concentration
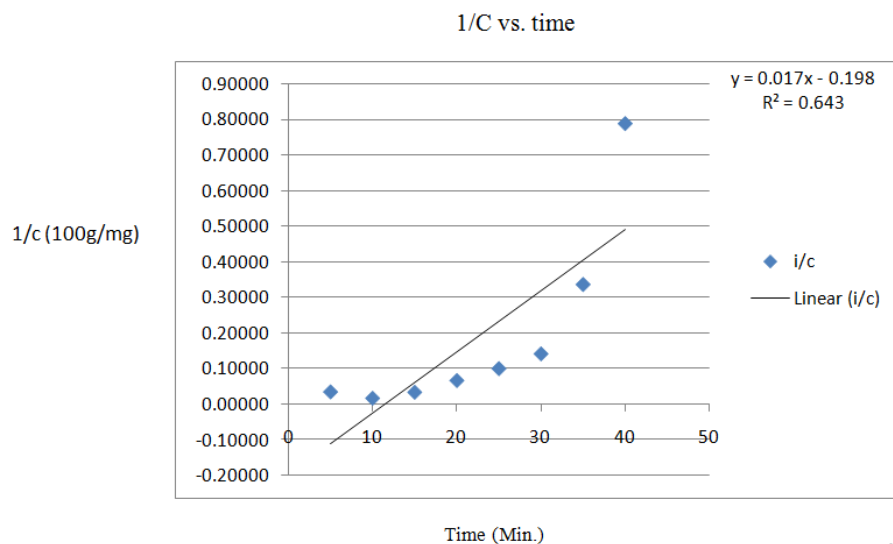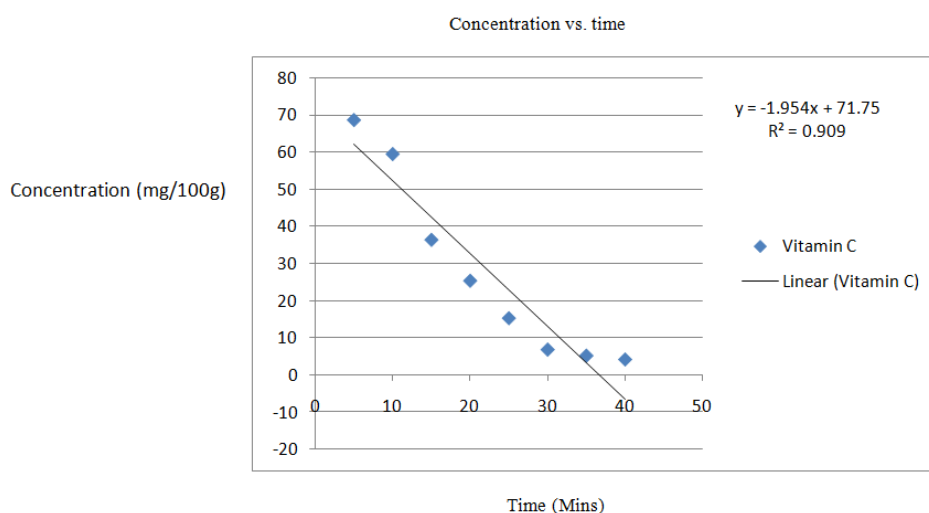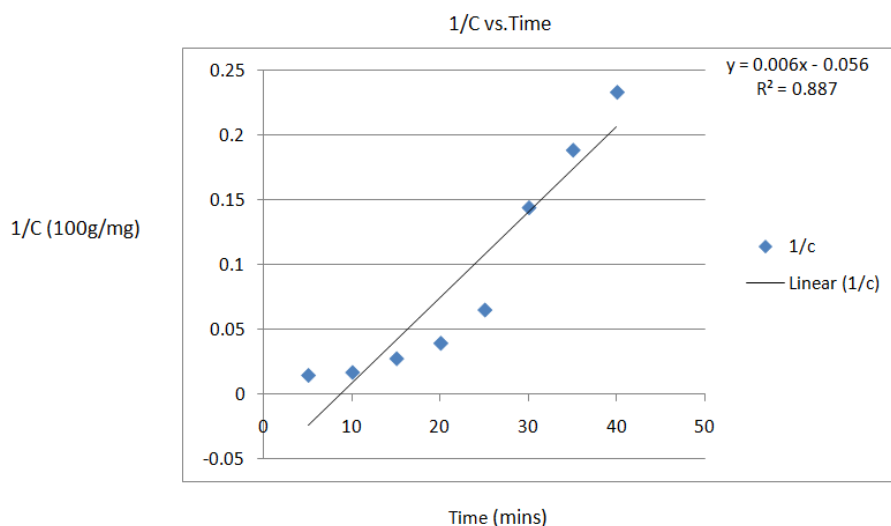
1/C vs. time

$y = 0.017x - 0.198$
$R^2 = 0.643$

Fig. 3. A plot of second order kinetics for Lettuce (1/C vs. time)

Concentration vs. time

$y = -1.954x + 71.75$
$R^2 = 0.909$

Figure 4. A plot of zero order kinetics for Cabbage (concentration vs. time)

ln (C) vs. time

$y = -0.088x + 4.849$
$R^2 = 0.978$
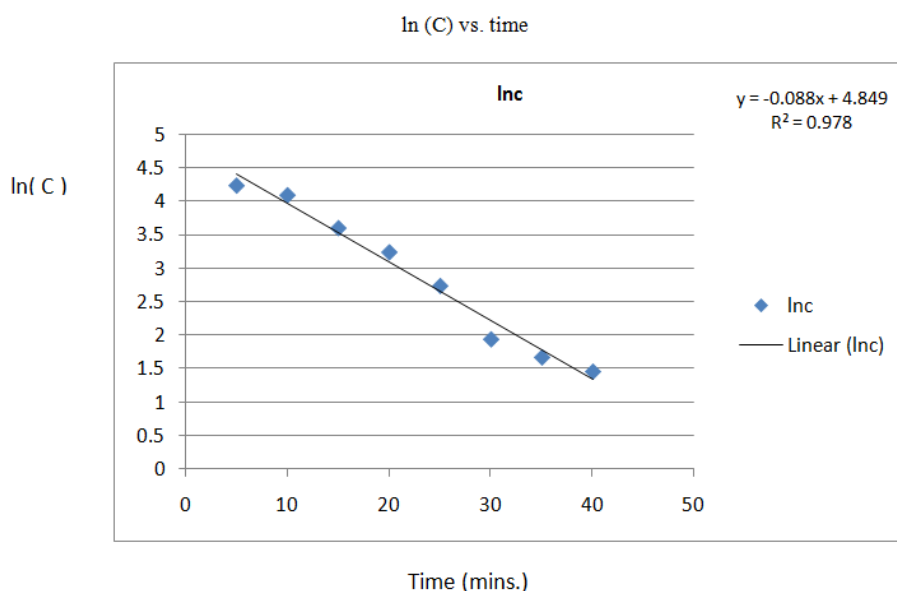
Fig. 5. A plot of first order kinetics for Cabbage (ln C vs. time)

Fig. 6. A plot of second order kinetics for Cabbage (1/C vs. time)

The lettuce and cabbage salad vegetable samples exhibited rate constants of 0.099min-1 and 0.088 min-1 respectively, of which cabbage was the lowest. Since the magnitude of the rate constant is a reflection of the rate of reaction, the inference is that degradation of vitamin C occurred faster in Lettuce sample than Cabbage [31]. This trend manifested in the half life of the samples which gives further credence to this fact. The time at which the concentration of vitamin C in the Lettuce sample reduces to half of its original amount (half life) was shorter than in Cabbage sample. This implies that the Cabbage sample will be expected to have longer shelf life than the Lettuce. Cabbage salad vegetable sample recorded the longest half life of 472.6004seconds while Lettuce had the shortest half life of 420.0892seconds.The mean value quantified individual models, for first order, the mean value was 2.4049 for Lettuce and 2.8697 for Cabbage, which implies that the higher the mean value for specified model the lesser the rate of degradation.ln(c) forecast; 1.3300 for Cabbage and ln(C); 0.6580 for Lettuce, which further authenticate that blanched Lettuce degradation was at higher rate than Cabbage under the same pretreatment conditions.

IV. Conclusion

The rate of vitamin C degradation in the Lettuce and Cabbage samples under the same processing method investigated in this study followed a first order reaction kinetics. This indicates that the rate of degradation is dependent on the concentration of the vitamin C present in both vegetables. In addition, pretreatment of salad vegetable (Lettuce) resulted in faster rate of degradation of vitamin C than vegetable Salad (Cabbage). This impresses the fact that the blanching of Cabbage at 70^0 C of water is more preferable in terms of vitamin C retention than Lettuce, hence, is recommended for food supplement Industries to deploy this research findings for further processing into semi and finished products after dehydration because degradation of vitamin C during processing might be a critical factor for the shelf life of some products such nutrient concentrates, since vitamin C content of fruits undergoes destruction during processing and storage.

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