JOTCS A
Journal of the Turkish Chemical Society
Section: A
Chemistry
TURKISH CHEMICAL SOCIETY

Forecasting the Degradation of Vitamin C in Commonly Consumed Vegetable Cabbage (*Brassica oleracea*) Dipped in Different Pre-treatment Solutions

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Abstract: Vitamin C is considered an essential vitamin that is commonly found in most fruits and vegetables. It is susceptible to easy degradation, especially during pre-treatment and storage. This study aimed at forecasting the degradation of vitamin C in commonly consumed vegetable (cabbage) dipped in different pre-treatment solutions at different time intervals. The samples after dipping at different time intervals were prepared for analysis. Evaluation of the ascorbic acid content of the vegetable was determined using High-Performance Liquid Chromatography (HPLC). This consists of an isocratic elution procedure with ultraviolet-visible detection at 245 nm. The half-lives and rate constants were calculated using the integrated law method. Forecasts were determined using time series analysis. Degradation of vitamin C in this study followed a first-order kinetic model, and the average coefficient of determination (R^2-value) was greater than 0.9413. The rate constants of vitamin C degradation for the vegetable dipped in different pretreatment solutions (sodium chloride, sodium benzoate, sodium metabisulfite and vinegar) at different time intervals were 0.0804, 0.1049, 0.0706 and 0.0553 minutes^{-1}; half-lives were 8.2322, 7.3896, 10.9675, and 12.1086 minutes, respectively. The vegetable dipped in different pretreatments for 90 minutes exhibited ln(C) forecast of -2.2057, -4.6307, -1.1746, and 0.0789, respectively. The coefficient of correlation for sodium chloride, sodium benzoate, sodium metabisulfite, and vinegar are 0.084, 0.093, 0.063 and 0.059 respectively. The kinetic models were formulated using predicted initial contents, processing time, and measured contents. The vegetable dipped in vinegar pretreatment solution using $\ln(C) = \ln(C_0) - 0.0553t$ gave the best model. From the results, the most appropriate pretreatment solution for enhancing the shelf life of cabbage is synthetic vinegar (prepared from acetic acid) because it has a lower rate constant, lower coefficient of correlation, and the longest half-life.

Keywords: HPLC, Cabbage, Pretreatment Solutions, Ascorbic Acid, Rate Constant, Forecast

S**ubmitted:** March 31, 2022. **Accepted:** January 12, 2023.

Cite this: Awagu EF, Okoroafor HC, Okike OO, Daramola DS, Arohunmolase OM. Forecasting the Degradation of Vitamin C in Commonly Consumed Vegetable Cabbage (*Brassica oleracea*) Dipped in Different Pre-treatment Solutions. JOTCSA. 10(1):109–16.

DOI: https://doi.org/10.18596/jotcsa.1093112.

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1. INTRODUCTION

Vitamin C is water-soluble and is a very important component of a healthy meal (1, 2). The increasing demand for nutritious food has led to several attempts to maximize the retention of nutrients in both processed and stored foods. Vitamin C serves as a quality indicator for other food nutrients especially during processing and storage of foods because, if vitamin C is well retained, the other nutrients are also well retained (1). Vitamin C is the most unstable vitamin, which easily deteriorates during processing and storage. This can be because of certain variables such as pH (3, 4), temperature (5, 6), light (7, 8), the presence of enzymes (9, 10), hydrogen peroxide (11), and metallic catalysts (12-14).

Cabbage is a very essential and highly rated leafy vegetable, which contains minerals, vitamins, and alkaline salts. It is medicinal because of its wonderful cleansing properties such as the detoxification of reactive oxygen species (ROS) in the body (15). It contains tartronic acid which inhibits fat formation and other elements which enhance the body's immunity, slows down aging, inhibits cancer formation, and estrogenic activities. Cabbage is a very good source of vitamin C (16). The body cleansing properties of cabbage is because of a high content of ascorbic acid and sulfur-containing glucosinolates, anthocyanins, flavonoids, and other useful plant metabolites (17). Hydrolytic products of glucosinolates are responsible for the prevention of oxidative stress, induction of detoxification enzymes, stimulation of the immune system and the reduction of cancer risk (18).

Sodium chloride solution inhibits polyphenol oxidase (PPO), thereby preventing browning of fruits and vegetable products (19). Benzoic acid and its salts are very common preservatives, which inhibits an enzyme in the citric acid cycle of microorganisms. These pretreatment solutions function effectively under highly acidic conditions. They inhibit the activities of yeasts, mold, and microorganisms producing aflatoxins, but are not too effective against bacteria (20). The chemical preservative such as sodium metabisulfite is effective in the control of pathogenic bacteria causing food poisoning and infection (21). Research revealed that vinegar had the highest effect on the bacterial load of vegetables. This might be due to the acidic pH of vinegar, and some microorganisms cannot withstand acidic conditions (22).

Thermal processing is a traditional method for pretreating fruits and vegetables, and this includes drying, cooking, and blanching. However, ascorbic acid (vitamin C) is one of the key vitamins and it is highly susceptible to degradation and this is influenced by several factors such as high temperature, oxygen, and light (23, 24). Understanding the mechanism through which vitamin C is degraded during food processing is an essential tool in the regulation of the parameters needed to improve vitamin C retention. Additionally, understanding the degradation kinetics and various kinetic models is crucial in forecasting vitamin C loss and change in quality under certain processing conditions. Kinetic modeling enables us to quantify these changes and the rate at which they occur. It also aids us in understanding fundamental reaction mechanisms required for quality control and modeling. Vitamin C degradation kinetics in modeled systems follows first-order kinetics; but is very complex in food systems (25). This complexity as observed in food systems hinders the formulation of suitable mechanistic models. To get a good fit for the experimental data, pseudo-kinetic models, such as zero-order, first-order, or second-order kinetics, are commonly used. The model that produces the highest coefficient of determination value (R^2) is considered the best fit for the analysis.

A time series analysis comprises of formulating a model that represents a time series and deploying the same in forecasting future values. The present study aimed at determining the rate of degradation of vitamin C in cabbage dipped in different pretreatment solutions, to recommend the best; development of kinetic models for forecasting the degradation of vitamin C in cabbage under the studied conditions; and to forecast future values.

2. MATERIALS AND METHODS

Metaphosphoric acid, L-ascorbic acid, orthophosphoric acid, and acetonitrile (High-Pressure Liquid Chromatography (HPLC)-Grade) were all purchased from Merck (Darmstadt, Germany). De-ionized water of 18 MΩ cm^{-1} resistivity purified with a milli-Q system (Millipore, Bedford, MA, USA) was used for chromatographic analysis; ascorbic acid stock standard solution kept inside a glass-stoppered bottle (26).

Freshly cut vegetable (cabbage) weighing 4.5 kg, was washed with clean water, and drained using a clean muslin cloth. Stems were removed with a clean, sharp knife. The weighed cabbage leaves of 4.5 kg were divided into 9 lots of 100 g each and dipped differently into 2 liters of water containing 0.6 g each of sodium chloride (27.3±0.92 °C), sodium benzoate (26.8±0.64 °C) and sodium metabisulfite (27.9±0.18 °C) for 5, 10, 15, 20, 25, 30, 35 and 40 minutes, respectively. Also 100 g of the cabbage was dipped into 2 liters of water containing 100 mL of vinegar at various time intervals. The dipped leaves at the completion of time in the pretreatment solutions were immediately ran under running tap water for 30 seconds to stop further effects of the pretreatments. They were then drained differently for 2 minutes using a clean muslin cloth. The initial sample was blended using a Kenwood blender (Philips, HR 1702, Borehamwood, England, UK) and then filtered using cheese cloth. HPLC was used to determine the initial ascorbic acid degradation using the liquid extract, before dipping into pretreatment solutions. 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 1.2 cm^3min^{-1} and an injection volume of 20 µL was used in the quantitative analysis. The temperature of analytical column was kept constant at 25 °C. The remaining samples were spread evenly on labeled trays and kept under a room temperature range of 25 – 29 °C and relative humidity range of 50 – 61 % using a thermometer and hygrometer. The sample without any form of treatment was used as the experimental control. At the end of the drying period, the samples were blended, sieved, and the liquid extract (1 g of each sample to 25 mL of extractant containing 5 % metaphosphoric acid

(MPA) at 10 °C and in the dark) was used in determining the final rate of degradation of ascorbic acid. All extractions were carried out in triplicate and obtained solutions were then filtered and stored at 4 °C before analysis. The injection of the extracts into HPLC system was performed twice.

2.1. Kinetic Modeling

Integrated rate law was used in modeling the degradation of vitamin C and various models were formulated using the integral method of analysis. The integral law equation is as follows (27):

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

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

Zero order model (n = 0):

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

$$t_{\frac{1}{2}} = \frac{C_0}{2k} \quad (3)$$

First order model (n = 1):

$$\ln(C) = \ln(C_0) - kt \quad (4)$$

$$\left(t_{\frac{1}{2}}\right) = \ln\frac{(2)}{k} \quad (5)$$

Second order model (n = 2):

$$\frac{1}{c} = \frac{1}{C_0} + kt \quad (6)$$

$$t_{\frac{1}{2}} = \frac{1}{kC_0} \quad (7)$$

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

3. RESULTS

Table 1 presents concentrations (mg/100 g) of vitamin C in cabbage dipped in different time intervals in sodium chloride, sodium benzoate, sodium metabisulfite, and vinegar, respectively, indicating a decrease in vitamin C with an increase in time in all the pre-treatment solutions.

Table 2 shows that R^2 and R^2 adjusted increased in value as P –values decreased with increasing time. The p-value continually tends to zero as the R^2 value increases. This implies that vitamin C degradation kinetics for cabbage dipped in different pre-treatment solutions may be best described by first order kinetics.

Table 3 shows pretreatment solutions, temperature of the pre-treatments, rate constants, half–life, and proposed model. The lower the rate constant, the lower the degradation of vitamin C.

Table 1: Vitamin C degradation in cabbage dipped in different pretreatment solutions.

Time (min)	NA Vit.C (mg/100 g)	SB Vit.C (mg/100 g)	SM Vit.C (mg/100 g)	VIN Vit.C (mg/100 g)
5	128.80	37.23	80.01	150.09
10	80.81	31.57	69.77	75.79
15	35.08	28.92	59.78	68.46
20	27.43	14.78	48.69	54.89
25	20.73	11.47	35.97	45.23
30	19.19	6.99	25.77	37.86
35	9.37	2.89	17.25	21.08
40	5.16	1.41	7.33	14.25

n = 3 (triplicate), NA: sodium chloride SB; sodium benzoate, SM: sodium metabisulfite, VIN: vinegar

Table 2: Kinetic model regression analysis for cabbage dipped in different pretreatment solutions.

Pretreatment	R^2	R^2 Adjusted	P-value
NA	0.946726	0.936071	0.000227
SB	0.958414	0.950097	0.000122
SM	0.925188	0.910226	0.000534
VIN	0.934826	0.921791	0.000377

Table 3: Rate constant kinetic model regression analysis for cabbage dipped in different pretreatment solutions.

PT	Temp (°C)	k (min^1)	Half-Life (min)	Proposed model
NaCl	27.3 ± 0.92	0.0804	8.2322	$\ln(C) = \ln(C_0) - 0.0804t$
SB	26.8 ± 0.64	0.1049	7.3896	$\ln(C) = \ln(C_0) - 0.1049t$
SM	27.9 ± 0.18	0.0706	10.9675	$\ln(C) = \ln(C_0) - 0.0706t$
VIN	27.4 ± 0.19	0.0553	12.1086	$\ln(C) = \ln(C_0) - 0.0553t$

Figure 1 presents first-order kinetics for cabbage dipped in different pre-treatment solutions, showing that the rate of degradation at any time is dependent on the initial concentration of vitamin C in the vegetable.

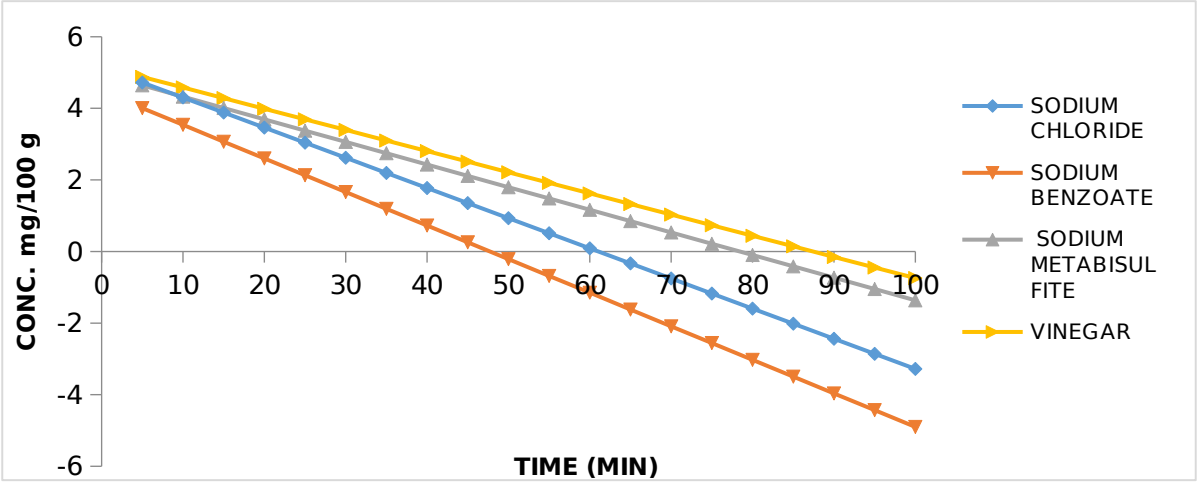

Figure 1: Plot of first-order kinetics for cabbage dipped in different pretreatment solutions.

Table 4: First-order kinetics Trendline equation and R Squared value for cabbage dipped in different pretreatment solutions.

Cabbage Dipped in Solutions	Y intercept	R Square
Sodium Chloride	-0.084x + 5.143	0.963
Sodium Benzoate	-0.093x + 4.474	0.939
Sodium Metabisulfite	-0.063x + 4.956	0.913
Vinegar	-0.059x + 5.171	0.953

The coefficient of correlation for sodium chloride, sodium benzoate, sodium metabisulfite and vinegar are 0.084, 0.093, 0.063 and 0.059 respectively (Figure 1). 0.059<0.063<0.084 <0.093. This implies that vitamin C in cabbage dipped in vinegar solution was more stable than vitamin C in cabbage dipped in the other pre-treatments.

Figure 2 presents cabbage dipped in different pre-treatment solutions and the forecasted shelf–life. Cabbage dipped in vinegar was preferred among others. From the time series forecast analysis, it can be deduced that vitamin C is more stable in cabbage dipped in vinegar solution because it has the least coefficient of correlation (0.059).

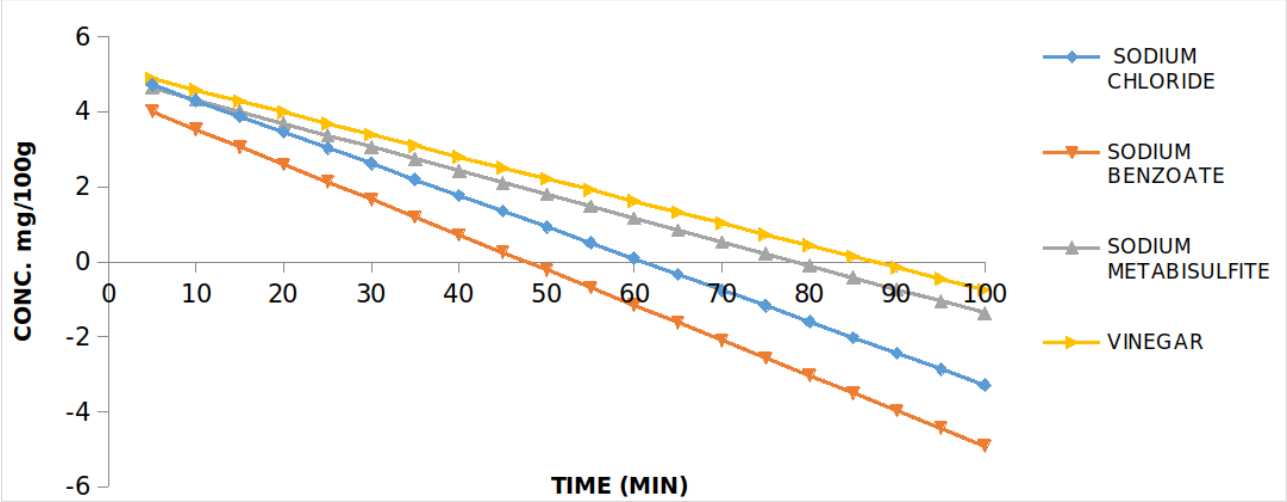

Figure 2: Plot of time series forecast analysis for cabbage dipped in different pre-treatment solutions.

Table 5: First-order kinetics Forecast Trendline equation and R Squared value for cabbage dipped in different pretreatment solutions.

Cabbage Dipped in Solutions	Y intercept	R Square
Sodium Chloride	-0.084x + 5.143	1
Sodium Benzoate	-0.093x + 4.474	1
Sodium Metabisulfite	-0.063x + 4.956	1
Vinegar	-0.059x + 5.171	1

4. DISCUSSION

Table 1 shows the variations in ascorbic acid concentration of cabbage dipped in different pretreatment solutions at different time intervals during processing and storage. Table 2 is a summary of kinetic model regression analysis results, and Table 3 shows vitamin C first-order kinetics degradation of cabbage dipped in the different pretreatment solutions. In Table 1, it was observed that the concentration of vitamin C in cabbage steadily decreased with increasing time. This reveals that vitamin C in the vegetable dipped in different pretreatment solutions degrade with time during processing and storage. Vitamin C concentration of cabbage decreased with time during processing and storage, but in varying degrees, and this was influenced by the different processing and storage methods (25). This concurs with some previously reported research findings on citrus and strawberry fruit juices (28, 29), and it also conforms to the degradation kinetics of vitamin C in model systems (16). The kinetic plots of the model (Eq. 4) in Figure 1 reveal that the first-order model was the best fit at different times. The goodness of fit data in Table 2 confirms this. Their R^2 values were the highest and *p*-values the lowest with different pretreatments. The first-order kinetic model showed a good fit for vitamin C degradation of most materials dipped in NaCl and has been utilized by several researchers (30-33). This was confirmed for NA from Table 2, R^2: 0.946726, *p*-value: 0.000227. Thus, first-order kinetics best describes the vitamin C degradation kinetics in the vegetable dipped in 0.3 g/L of each sodium chloride, sodium benzoate, sodium metabisulfite and 50 mL/L of vinegar pretreatments. The model with maximum R^2 and the minimum *p*-value is considered the best (34, 35). Furthermore, from Table 3, the rate constant of the vegetable sample dipped in vinegar exhibited the lowest rate constant of 0.0592 $minutes^{-1}$, indicating less degradation. Since the magnitude of the rate constant reveals the rate of reaction, it therefore shows that degradation of vitamin C occurred faster in those samples dipped at an increased time. In other words, the rate of degradation at any given time depends on the initial concentration of vitamin C in the vegetable. Additionally, the processing pretreatment solutions had an average mild temperature of 27.35 °C as shown in Table 3. An increase in storage temperature is therefore believed to result in an increase in the losses of vitamin C in the stored products (36, 5, 23, 37). The post-harvest losses of vegetables can be greatly reduced by extending their shelf-life (8). This trend as clearly revealed in the half-life of the samples in Table 3 supports these facts. The cabbage vegetable dipped in vinegar had the longest half-life of 12 minutes and 11 seconds. The product shelf life is often determined by the decrease of ascorbic acid concentration to industrially unacceptable levels (38). Again, from Figure 2, the ln(C) forecast

dipped for 90 minutes and exhibited -2.2057, -4.63069, -1.17463, and 0.078902 showing that vinegar richer in vitamin C. From Table 3, the kinetic models formulated using the predicted initial contents, the processing time, and measured contents, dipped in vinegar pretreatment solution with the model; $\ln(C) = \ln(C_0) - 0.0553t$ gave the best model.

Ascorbic acid is often used as the most common nutrient loss indicator during storage. The retention of ascorbic acid in food products is a reliable representative index during the processing of foods (36, 24). The proposed simulation model monitors the degradation of vitamin C (ascorbic acid) in cabbage and expresses the advantages of the computer simulation technique over the laboratory chemical analysis.

5. CONCLUSION

This work developed models in which the experimental variable can be imputed, and the desired results were computed for a better understanding of the vitamin C values and to make accurate predictions of different outcomes, regarding their shelf-life. Additionally, the models enable appropriate forecasting in agricultural produce. It also produces value-added products by slowing down or inhibiting chemical deterioration and microbial growth. All these absolutely support and authenticate that rate constant is the major determining factor in accessing the degradation of commonly consumed vegetables, which cabbage dipped in vinegar signified lesser degradation. The retention of ascorbic acid in cabbage after dipping in vinegar pre-treatment solution is a suitable indicator for the retention of other nutrients in cabbage.

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