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COMPARATIVE ANALYSIS OF THE RATE OF VITAMIN C DEGRADATION IN LETTUCE (*LACTUCA SATIVA*) TREATED WITH VARIOUS PRETREATMENT SOLUTIONS

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ABSTRACT

Lettuce is a highly perishable leafy vegetable that faces significant postharvest challenges, including water loss, browning, microbial contamination, and ethylene sensitivity. These issues lead to reduced shelf life, economic losses, and food waste, affecting both farmers and consumers. This study aimed to establish a kinetic model for the breakdown of ascorbic acid in lettuce via computer simulations. The vegetable samples were examined using high-performance liquid chromatography (HPLC) to assess the ascorbic acid (AA) content of the vegetables after they were dipped at various time intervals. This consists of an isocratic elution procedure with ultraviolet-visible detection at 245 nm. The average coefficient of determination (R^2-value), was greater than 0.9088, indicating that the AA degradation in the experiment followed a first-order kinetic model. Using the integrated law approach, rate constants of 0.0135, 0.0460, and 0.0341 k (min^{-1}) and half-lives of 51.3442, 15.0684, and 20.3269 minutes for lettuce dipped in NaCl, SB, and SM, respectively were determined. The Arrhenius equation was used to calculate the activation energies of 161.5341, 84.2009, and 48.6334 kcal/mol. Time series analysis was used to predict the amount of vitamin C at point "70" (Y-INTERCEPT), which was 2.3167, 0.1438, and 0.9561 mg/100 g. In(C): 6.800551, 6.570627, and 6.630127 were obtained when the kinetic models were constructed using the expected initial concentration, processing time, and observed contents respectively. This suggests that In(C) is directly influenced by the initial vitamin C concentration, with an In(C_o) concentration of 898.41 mg/100 g, a rate constant, and time. The best result was obtained by dipping the vegetable for five minutes in a pretreatment solution of 0.3 g NaCl/litre of H_2O; using the proposed model $\ln(C) = \ln(C_o) - 0.0135t$.

Keywords: Vitamin C, Degradation, Kinetics, Lettuce, Pretreatment

INTRODUCTION

Lettuce is the most extensively cultivated and consumed vegetable in the world (Medina-Lozano *et al*. 2021; FAOSTAT, 2021) with an increasing annual production rate. This plant, which is Mediterranean in origin and a member of the Asteraceae family, is prolific and diverse (Shi *et al*. 2022). Due to its therapeutic qualities, lettuce was originally grown as early as 26080 BCE. In addition to being particularly rich in vitamins, minerals, and bioactive substances (carotenoids, polyphenols, and chlorophyll) with linked health advantages, it has a low calorific value and a high moisture content (94–95%) (Yang *et al*. 2022). Moreover, lettuce has a remarkably high vitamin C content (Medina-Lozano *et al*. 2021), which further enhances its health benefits. Ascorbic acid, tocopherols, glycosylated flavonoids, phenolic acids, carotenoids, vitamin B groups, and sesquiterpene lactones are a few of the phytochemicals found in lettuce and are essential natural sources of bioactive nutritional components (Uddin *et al*. 2020; Mitra *et al*. 2022; Mitra *et al.* 2022b). Due to restrictions on the use of dangerous synthetic antioxidants and increased knowledge of the value of a healthy diet, natural antioxidant chemicals that have been isolated and discovered from plant sources, such as lettuce, have gained popularity (Vázquez *et al*. 2021).

Native green leafy vegetables play a significant role in soups and sauces that are paired with staple carbohydrates. Due to their high concentrations of flavonoids, phenolics, iron, calcium, protein, carotenoids, and vitamin C, they are recommended for the treatment of malnutrition (Musinguzi *et al*. 2011; Smith & Eyzaguirre, 2007). One way to determine whether additional nutrients are retained in these veggies is to use the ascorbic acid content, which is thought to be the most unstable component. The presence of numerous elements, such as temperature, pH, light, moisture content, and others, might affect the stability of vitamin C (Khan *et al*. 2006; Hiatt et al. 2010; Gundeshi *et al*. 2019; Mohammed *et al*. 2023).

Although lettuce has a high nutritional value, its shelf-life after harvest is extremely limited. Both biotic and abiotic variables are responsible for this phenomenon (Peng & Simko, 2023). Hence, this research aimed to evaluate the effects of pretreatment on the shelf-life of lettuce using vitamin C as a refractive index for nutrient retention. Three pretreatments, sodium chloride (NaCl), sodium benzoate (SB), and sodium metabisulfite (SM), were used for the study. Sodium chloride is a widely used food preservative that inhibits the growth of a variety of microorganisms. Sodium benzoate is a sodium salt that is present in many different goods, including sauces, juices, preserves, and beverages. It is commonly used as an additive in various cosmetic, medicinal products and culinary products (Lennerz *et al*. 2015). It has the chemical formula $C_7H_5O_2Na$, has an odourless molecule, and is soluble in water and ethanol (Linke *et al*. 2018). It is utilized in the pharmaceutical industry to treat a wide range of illnesses, including multiple sclerosis, liver conditions, and urea cycle problems (Yayav *et al*. 2016). Sodium benzoate is utilized in food products because, in addition to being simple to use, it effectively prevents the emergence of bacteria and fungus during storage (Tsay *et al*. 2007). According to Zhang and Ma (2013), it can be used to preserve margarines, sauces, marmalades, gelatin, liqueurs, beers, fruit juices, and soft drinks. Population studies show that soda and juice in cartons are the primary dietary sources

of this additive, albeit its prevalence in a variety of foods (Pongsavee, 2015). Its purpose is to prevent the growth of bacteria, yeasts, and mould (Turkoglu, 2007). Sodium benzoate is also regarded as safe by the Food and Drug Administration (FDA) (FDA, 2017).

According to Noorafshan *et al*. (2014), sodium metabisulfite (SM) is utilized as an additive, antioxidant, and bleaching agent in a variety of industries, including food, beverages, cosmetics, medicine, photography, and rubber. According to Sadowska *et al*. (2021), it is highly significant for the preservation of goods such as wine, beer, grapefruit juice, dried fruits, and seafood. It is also widely used in the production of processed foods. In addition to being widely used in the wine industry, sodium metabisulfite ($Na_2S_2O_5$) is an efficient preservative with well-known bactericidal qualities. It also works to prevent enzymatic and nonenzymatic browning reactions, effectively suppresses microbial growth, and preserves dry and fresh fruits. For instance, SM works well to prevent microbes from degrading olives and causing them to brown (Arroyo-López *et al*. 2008; Echevarria *et al*. 2010).

Understanding the various kinetic models and the kinetics of degradation is essential for predicting changes in lettuce quality and vitamin C loss under specific storage conditions. Therefore, it is necessary to make precise mathematical predictions about how ascorbic acid will behave during storage (Zhang & Lu, 2011). Numerous researchers have employed the first-order kinetic model because it has shown an excellent fit for the ascorbic acid degradation of most materials during storage (Zhang & Lu, 2011; Sapei & Hwa, 2014; Thuy *et al*. 2020; Soceanu *et al*. 2020). Hence, this study aims to establish a kinetic model for the breakdown of ascorbic acid in lettuce via computer simulations.

MATERIALS AND METHODS

Reagents and Chemicals

Merck (Darmstadt, Germany) provided the orthophosphoric acid, metaphosphoric acid, and acetonitrile high-pressure liquid chromatography (HPLC grade) reagents. A Milli-Q system (Millipore, Bedford, USA) was utilized to purify deionized water with a resistivity of 18 MΩ cm^{-1}, which was then used for chromatographic analysis. A glass stopper bottle containing the standard AA stock solution was kept at 4 °C in the dark after being made in water (Awagu *et al*. 2017).

Sample preparation

Mature and fresh lettuce was obtained from Yankaba, Nasarawa Local Government of Kano State, Nigeria, which is a fruit and vegetable market. A muslin towel was used to drain 9 kg of the fresh carrots after they had been thoroughly cleaned with clean water to remove any remaining water. Using a clean, sharp knife, the stems were chopped off to prevent infestation. After being separated into nine batches of 100 grams each, the vegetables were immersed in two-liter volumes of water comprising 0.6 grams of sodium chloride (27.3±0.92 °C), sodium benzoate (26.8±0.64 °C), and sodium metabisulfite (27.9±0.18 °C) for five, ten, fifteen, twenty, thirty, and forty minutes, respectively. To prevent the effects of the pretreatment from continuing, the immersed leaves were promptly rinsed under running tap water for 30 seconds after they were removed from the solutions. After that, a fresh muslin cloth was used to drain them separately for two minutes. The original sample was filtered through a cheesecloth after being blended in a Kenwood blender (Philips, HR 1702, Boreham wood, England, UK). Before the samples were dipped into pretreatment solutions, HPLC was utilized to measure the initial ascorbic acid breakdown of the liquid extract. Using a thermometer and hygrometer, the remaining samples were evenly distributed on marked trays and maintained between 25 and 29 °C at ambient temperature and between 50 and 61% relative humidity. The experimental control sample received no treatment at all. After the drying phase, the samples were mixed; and sieved, and the liquid extract (1 g of each sample to 25 ml of extractant containing 5% metaphosphoric acid (MPA)) at 10 °C in the dark) was used to calculate the ascorbic acid degradation rate. Before analysis, all extractions were performed in triplicate, and the resulting solutions were filtered and kept at 4 °C. Two extraction injections were made into the HPLC apparatus.

HPLC Analysis

The liquid chromatographic method was used to measure the concentration of AA using an isocratic elution procedure with UV visible detection at 245 nm. A 250 mm ˛ 4.6 mm spherical Optimal ODS-H column from Capital HPLC, UK was used for the experiments. It was outfitted with a 20 mm ˛ 4.6 mm spherical RP C18 guard column. The mobile phase was made up of acetonitrile (93:7) and 0.5% NaH_2PO_4 (pH 2.25 with H_3PO_4).

A 20 ml injection volume and a 1.2 ml/min mobile phase flow rate were employed for the quantitative analysis. A constant of 25°C was maintained for the analytical column temperature. The quantitative analyses and calibration curve concluded at 245 nm. To prevent AA loss, extracted samples, and standard solutions were kept out of the light using amber flasks. Standard solutions and extracts were filtered using a prefilter and a 0.45 mm Millipore membrane before injection. Quantification was possible through a comparison of the chromatographic peak region with the external standard. With a concentration range of 0.5–200 mg l^{-1}, the calibration curve was plotted using a 10-point calibration.

Kinetic modelling

Utilizing the integrated rate law, a model of vitamin C degradation was created. The integral approach of analysis was used to construct several models. The following is the integral law equation.

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

was employed to create three models that utilized half-lives ($t_{1/2}$) and concentrations (for reaction orders, n = 0, 1, and 2).

Zero-order model (n = 0):

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

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

First-order model (n = 1):

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

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

Second-order model (n = 2):

$$\frac{1}{c} = \frac{1}{\text{C0}} + kt \quad (4a)$$

$$t_{\frac{1}{2}} = \frac{1}{kC_O} \quad (4b)$$

where k = rate constant, C_o = 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.

Statistical analysis

For each model, the concentration or a function of concentration was plotted against time using Minitab 1723. Regression analysis was used to estimate the "goodness of fit" (Barbara *et al*. 2017). A statistical program was used to develop the kinetic model, which considers the observed contents, processing time, and expected initial contents (Bryman & Cramer, 2011). The coefficient of determination (R2) of a response variable indicates how well it matches the

data. Several comparisons of the treatment means were made using Duncan's unique multiple-range test. The data were analysed using analysis of variance (ANOVA) using the statistical programme SPSS 23.00 (2017) (SPSS Inc., Chicago, IL., USA). Differences that were considered significant were those for which p was less than 0.05, according to Duncan's unique multiple-range test.

RESULTS AND DISCUSSION

Table 1 shows that the rate of vitamin C degradation in the three pretreatment groups was significantly affected by time. The longer the duration of exposure of lettuce to these pretreatments was, the lower the vitamin C concentration was after exposure. The vitamin C content of lettuce was best retained in lettuce treated with NaCl, and most of the vitamin C content decreased in lettuce treated with sodium benzoate. It can be inferred from this result that NaCl is the best pretreatment for lettuce compared with sodium benzoate and sodium metabisulfite. As shown in Table 1, the concentration of vitamin C in lettuce decreased steadily with increasing time and temperature.

Table 1: Changes in the vitamin C content of lettuce vegetables dipped in pretreatment solutions over time

Time (mins)	Let(Vit. C) mg/100 g (NaCl)	Let(Vit. C) mg/100 g (SB)	Let(Vit. C) mg/100 g (SM)
5	16.48	17.11	20.08
10	25.72	16.46	19.46
15	26.57	15.56	18.57
20	24.14	13.11	16.51
25	20.37	12.94	13.73
30	18.28	10.76	10.21
35	16.17	5.59	8.58
40	12.28	2.75	6.18

n=3 (triplicates)
NaCl: sodium chloride, SB: sodium benzoate, SM: sodium metabisulfite

Treatment with NaCl had the lowest p-value, while the highest p-value was recorded for the lettuce samples treated with sodium benzoate (Table 2). As the R^2 value increases, the p value usually tends towards zero. It can be inferred from this that the vitamin C degradation kinetics for lettuce subjected to different pretreatments can be best described by a first-order reaction.

Table 2: Results of kinetic model statistical analysis for lettuce

Vegetable	Treatment	Temp (°C)	Statistics Parameters (First Order)		
			(R^2)	R^2 adjusted	P value
Lettuce	NaCl	27.36	0.9681	0.9618	0.00006205
Lettuce	SB	26.82	0.8028	0.7634	0.006328488
Lettuce	SM	27.5	0.9554	0.9465	0.0001448

n=3 (triplicate)

Compared with lettuce subjected to the other pretreatments, lettuce subjected to NaCl had the longest half-life (51 minutes, 3 seconds); and activation energy (E_A) (161.5341 kcal/mol) and the lowest rate constant (0.0135 min^{-1}). Lettuce plants dipped in sodium benzoate had the shortest half-life (15.06842) and the longest rate constant (0.046). This finding suggested that the degradation of vitamin C was greatest in lettuce dipped in sodium benzoate and much lower in lettuce dipped in sodium chloride.

Table 3: Rate constant kinetic model regression analysis for lettuce dipped in pretreatment solutions

Vegetable	PT	k (min^{-1})	Half-Life	E_A kcal/mol	Proposed Model
Lettuce	NaCl	0.0135	51.3442	161.5341	$\ln(C) = \ln(C_o) - 0.0135t$
Lettuce	SB	0.046	15.06842	84.2009	$\ln(C) = \ln(C_o) - 0.046t$
Lettuce	SM	0.0341	20.3269	48.6334	$\ln(C) = \ln(C_o) - 0.0341t$

The plot of the first-order reaction in Figure 1 reveals that the rate of degradation of vitamin C at any point in time is dependent on the initial concentration of vitamin C in the vegetable.

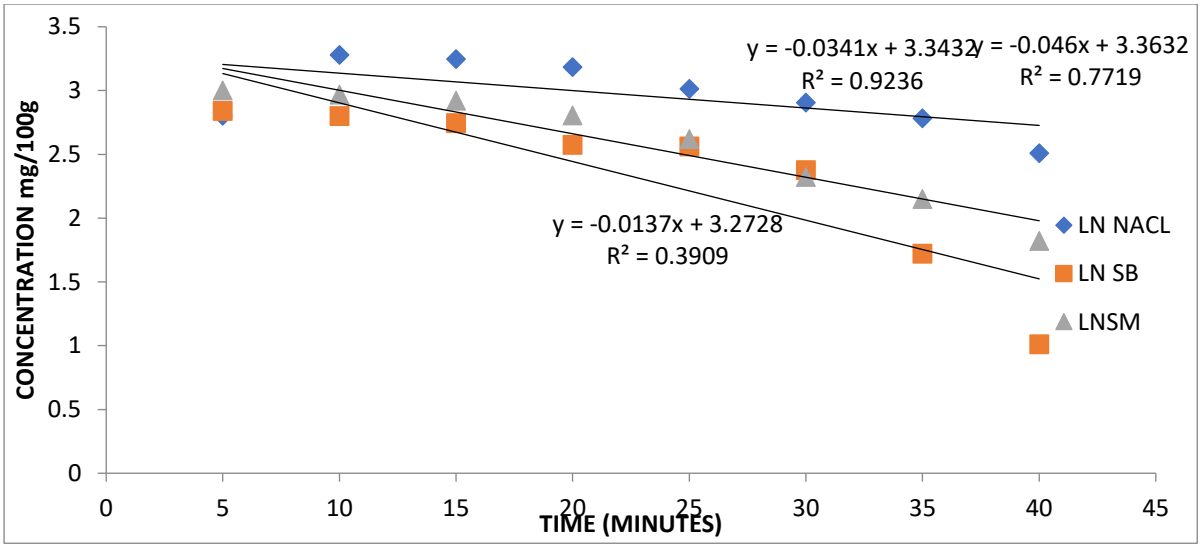

Figure 1: The plot of first-order kinetics for lettuce vegetables dipped in different pretreatment solutions

Figure 2 presents the time series forecast analysis of lettuce dipped in the three pretreatments (sodium chloride, sodium benzoate, and sodium metabisulfite). Lettuce dipped in sodium chloride was more preferred than lettuce dipped in other salts. From the forecast, it can be deduced that vitamin C was more stable in lettuce dipped in NaCl because it had the lowest rate constant of 0.013 (Figure 2). The vitamin C concentrations at point "70" (Y- INTERCEPT) were 2.3167, 0.1438, and 0.9561 mg/100 g according to the time series analysis. This refers to the vitamin C content of lettuce (*Lactuca sativa*) after 70 minutes of NaCl, SB, or SM treatment respectively. This finding implies that the vitamin C content in lettuce can be reduced beyond an experimental duration of 40 minutes.

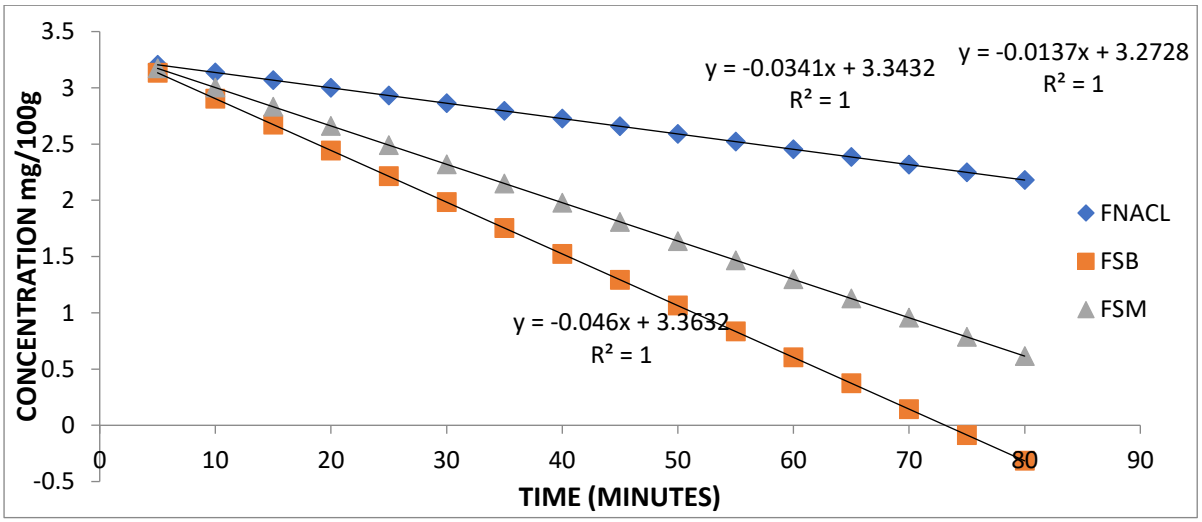

Figure 2: Plot of time series forecast analysis for lettuce vegetables dipped in different pretreatment solutions

The table below (Table 4) reveals the stability of vitamin C in lettuce subjected to pretreatment with the lowest stability in the presence of sodium benzoate and the highest stability in the presence of sodium chloride, with correlation coefficients of 0.046 and 0.013, respectively.

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

PT	Y-INTERCEPT	R-SQUARED VALUE
NaCl	0.013x + 3.272	0.390
SB	-0.046x + 3.363	0.771
SM	-0.034x + 3.343	0.923

With increasing time, a consistent decrease in the vitamin C concentration in lettuce was detected. This is consistent with the kinetics of vitamin C degradation in model systems (Hailemariam & Wudineh, 2020) as well as other previous research on the juices of strawberries and citrus fruits (Zhang & Lu, 2011; Frias & Oliveira, 2001). According to the results of the kinetic plot, the first-order model fits the kinetics of vitamin C degradation in lettuce the best. This may be inferred from the low p values and high R^2 values in Table 2 for each of the pretreatments. The first-order kinetic model has been used in several studies and is a good fit for the vitamin C degradation of most materials dipped in NaCl (Mitra *et al.*

2011; Silva *et al*. 2011; Leong & Oey, 2012; Wawire *et al*. 2011). Accordingly, first-order kinetics best describes the vitamin C breakdown kinetics in vegetables dipped in 0.3 g/L sodium chloride, sodium benzoate, or sodium metabisulfite pretreatments.

Table 3 shows how quickly vitamin C in lettuce deteriorates based on the activation energy and rate constants. The activation energy is the first energy barrier that any chemical reaction must overcome. Consequently, when the activation energy increases, the rate of reaction decreases. A lower activation energy suggests that a particular molecule breaks down more quickly with a smaller temperature shift, which is consistent with the range measured by Mauri *et al*. (1989) for ascorbic acid degradation in lettuce. This is because lettuce dipped in sodium chloride had the highest activation energy and the lowest rate constant among the pretreatment groups, it may be inferred from the data that lettuce dipped in sodium chloride best preserved vitamin C. The nutritional constancy of vegetables is indicated by their half-life. The reduction of vitamin C content to a level deemed insufficient by industry standards or declarations can be characterized as the product's shelf life. The longest half-life was observed in lettuce submerged in NaCl, clocking in at 51 minutes and 3 seconds. The results also indicate that the rate of vitamin C degradation at any given time is dependent on the initial concentration of vitamin C in vegetables (Fig. 1). The best model was obtained by dipping the kinetic models in a sodium chloride pretreatment solution with the model $\ln(C) = \ln(C_o) - 0.0135t$, which was created using the expected initial vitamin C concentration, the processing duration, and the observed contents (Table 3). A common biomarker of nutritional loss during storage is ascorbic acid. A reliable representative measure of food processing is the amount of ascorbic acid that food products retain (Burdurlu et al. 2006; Bhardwaj & Pardey, 2011). The proposed simulation model tracks the loss of vitamin C, or ascorbic acid, in lettuce and demonstrates the advantages of computer modelling over laboratory chemical analysis. According to the time series analysis, the vitamin C concentrations at point ‘‘70’’ in Table 4 (Y-INTERCEPT) were 2.3167, 0.1438, and 0.9561 mg/100 g respectively. Using the Y-intercepts for NaCl (0.013x + 3.272), SB (-0.046x + 3.363), and SM (-0.034x + 3.343) as references, we can obtain corresponding concentrations of 2.3167, 0.1438, and 0.9561 mg/100 g respectively, where X is the time (t) in this example, which is 70 minutes. The concentration of vitamin C after 100 minutes can be calculated similarly.

CONCLUSION

The rate of vitamin C degradation in lettuce under the particular processing and storage conditions examined in this study was investigated via first-order reaction kinetics. This implies that the rate of breakdown is influenced by the vitamin C concentration present in the vegetable at first. Furthermore, it can be inferred that pretreatment improves vitamin C preservation in vegetables, as evidenced by the rate constant, half-life, prediction, and activation energy. The best outcome, using the recommended model $\ln(C) = \ln(C_o) - 0.0135t$, was achieved when the lettuce was immersed in a 0.3 g NaCl/ 1 liter H_2O pretreatment solution for five minutes. According to the model equations, this is because of its longer half-life, higher activation energy, lower rate constant, and longer forecast. The model $\ln(C) = \ln(C_o)$ - (rate constant)t is suggested to minimize vitamin C losses during processing and storage.

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