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Kinetic Modelling of Vitamin C Degradation in Leafy Vegetables during Blanching

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Abstract: Three different commonly used leafy vegetables in Nigeria (*moringa oleifera, hibiscus esculentus* and *hibiscus sabdarifa*) were analysed for their ascorbic acid (Vitamin C) contents. The vegetables were blanched in a steam blancher for 1-6 minutes to analyse the effect of blanching time on their vitamin C content. *Hibiscus esculentus* had the lowest ascorbic acid content while *hibiscus sabdarifa* had the highest of Vitamin C concentration before blanching. After blanching for 6 minutes, *hibiscus esculentus, Moringa oleifera and Hibiscus esculantus* lost 69.7%, 64.2% and 54.2% of their initial Vitamin C contents respectively. Integral fitting of the experimental data shows that the kinetic degradation of ascorbic acid in all three vegetables follows first order reaction mechanism. The kinetic model parameters were determined using the integral method of data analysis for each vegetable. The pattern of ascorbic acid degradation in all the three vegetables was similar despite their different initial contents.

Keywords: Moringa Oleifera, Hibiscus Esculentus, Hibiscus Sabdarifa, Ascorbic Acid, Kinetic Modelling

1. Introduction

1.1. Background

Ascorbic acid, also called vitamin C, is a vitamin which requires particular attention from the practicing nutritionists. Unlike some B-group vitamins which are known to exist and play a part in the biochemistry of the body but of which a dietary shortage hardly ever seems to occur, ascorbic acid is quite often found to be deficient in people's diets, and their health may suffer when this is so. Low levels of vitamin C can result in a condition called scurvy. Scurvy may cause symptoms such as rash, muscle weakness, joint pain, tiredness, and tooth loss [1, 2]. Magnus P. [2] reported some reasons why ascorbic acid is lacking more often than perhaps any other nutrients:

a. It is only present in significant amount in a limited range of foods, particularly fresh fruits and vegetables.
b. It is promptly soluble in water and may be washed out of food in cooking water.
c. It is quite sensitive to heat and may be destroyed when vegetables are cooked or when they are kept hot for a period of time.
d. It gradually breaks down when vegetables are stored or when they become stale and wither.
e. It is not stored by body to any significant extent, so that a steady and adequate supply is necessary for complete health.

Although one may associate vitamin C with citrus fruits, ascorbic acid is found in a wide variety of plant tissues. With few exceptions, vitamin C is only found in fruits and vegetables. Furthermore, the amount of the vitamin C even in these may vary or may be lost altogether, they are only significant when the foods are fresh or have been carefully preserved or stored in the fresh form. Dried peas and beans and dried fruit do not contain any ascorbic acid [2]. Ascorbic acid is an excellent reducing agent and most likely acts in such a capacity in the plant cell. It is commonly associated with chloroplast and is present in high quantity in green leafy vegetables, as found in Roselle *(Hibiscus sabdarifa*), Okra *(Hibiscus esculentus L)* and drumstick leaves *(Moringa oleifera)*.

As a class, leafy vegetables are relatively rich in vitamin C and together with fruit, furnish most of the diet of urban families in most part of the world [3]. In Nigeria, as in most parts of Africa, leafy vegetables are consumed daily in the predominant diets and are grown in areas far from the urban areas. Even those grown relatively near are in the process of wilting by the time they appear on the market stalls, until they are bought for food. Apart from this, illumination of vegetable leaves has also been shown to lead to rapid oxidation of ascorbic acid [4]. This implies that handling conditions prior to cooking could also be important for the ultimate level of the vitamin in the cooked vegetables.

1.2. Handling and Processing of Leafy Vegetables

Among Nigerian communities, especially in the northern region, leafy vegetables are handled in various ways. Some of these handling and processing conditions include but are not limited to [4, 5];

a. cooking immediately after harvesting
b. cooking after leaving on the kitchen table for some time
c. cooking after leaving in a closed cupboard for some time
d. cooking after leaving in a refrigerator for some time
e. purchasing from market, where the vegetables must have been exposed to direct sunlight for some time, and cooking immediately or sometime later
f. Cooking after drying the leaves under direct sunlight.

Blanching or scalding is a physical process done with hot water or steam. The technical reason for blanching or using this operation is for enzyme inactivation, removing air from the tissue of the product and for controlling the fill into container. Since the main objective is to inactivate the enzyme responsible for causing off-flavour development during storage of frozen and dried produce, it is necessary to ensure that it is heated for a sufficient length of time at a temperature range of 91-99°C This will vary depending on the thickness of the produce as presented on table 1 below [6].

Table 1. *Blanching times for vegetables at 95°C [6].*

Products	Time (min)
Artichokes	5-9
Asparagus	2-5
Bean, green	2-3
Brussels sprouts	4-5
Carrots	2-5
Cauliflower florets	3-4
Celery	2
Corn –on-the-cob	6-11
Peas	1-2
Spinach	2

1.3. Motivation

The blanching time is very useful and varies with the vegetable type and size. Underblanching stimulates the activity of enzymes and is worse than no blanching. Overblanching causes loss of flavor, color, vitamins and minerals [7]. Fafunso *et al*., [3] reported losses in the vitamin C content of various vegetables as a result of drying, cooking, exposure to various environmental temperatures and blanching. But there is no records of vitamin C losses due to blanching at specific time and temperatures for most of the vegetables consumed in the Nigeria.

1.4. Description of Leafy Vegetables Studied in This Work

1.4.1. Moringa Oleifera

Moringa oleifera, commonly referred to simply *Moringa* is the widely cultivated variety of the genus *Moringa.* It is of the family *moringaceae.* It is an exceptionally nutritious vegetable tree with a variety of potential uses. The tree itself is rather slender with drooping branches that grows to approximately 10m in height; however, it normally is cut back annually to 1m or less, and allowed to re-grow, so that pods and leaves remain within arm’s reach [8]. Moringa oleifera is a fast-growing tree native to South Asia and now found throughout the tropics. Its leaves have been used as part of traditional medicine for centuries, and the Ayurvedic system of medicine associates it with the cure or prevention of about 300 diseases [9,10]. According to Abdull Razis *et al.,* [9], Moringa leaves are loaded with vitamins, minerals, essential amino acids, and more. One hundred gram of dry moringa leaf contains:

a. 9 times the protein of yogurt
b. 10 times the vitamin A of carrots
c. 15 times the potassium of bananas
d. 17 times the calcium of milk
e. 12 times the vitamin C of oranges
f. 25 times the iron of spinach

1.4.2. Hibiscus **Sabdarifa**

Hibiscus sabdarifa belongs in the family malvaceae. The common name includes Roselle, Red sorrel, Jamaican sorrel, Indian sorrel or sorrel sour- sour, yakuwa etc. *Hibiscus sabdarifa* is probably native to tropical central or West Africa. It was being cultivated in Asia over 300 years and was introduced into Brazil in the seventeenth centaury. It is also recorded as being known in the West Indies from the early eighteenth century; now widely distributed throughout tropical areas. In Nigeria, it is abundantly found in the northern region. *Hibiscus sabdarifa* is an erect, branched woody annual plant, up to 3m in height. The stem is red branched. Fruits has an oval capsule up to 3cm in length containing 15-25 seeds per capsule. The seeds are dark brown, and about 4-6mm long. Roselle has a variety of uses; the young shoots and leaves are eaten either raw or as cooked vegetables; the swollen calyces of the flowers are used in the preparation of beverages preserves and jellies. The stem has an economic fibre used in textile industries. And the seeds are of economic importance containing 18-22% oil [11, 12].

1.4.3. Hibiscus Esculentus

This is also included in the family of malvaceae. The common names include Okra, Ochra, Okro, and lady's finger. Origin of *Hibiscus esculentus* is probably tropical Africa or possibly tropical Asia. It was known in Egypt in the twelfth to thirteenth century AD, now widely distributed in many tropical areas. It is abundantly found in all regions of Nigeria unlike Roselle. Okra is an erect, hairy, woody annual herb, up to 2m in height. The fruit has a capsule about 20-30 seeds which are dark green to grey-black, rounded with cruncle. Okra is food valued highly by consumers. The immature fruit, when boiled or fried contains a large quantity of glycons which are responsible for the viscosity [13] and the viscosity decreases as temperature increases. The fruit is popular for adding to soups and stews. Young shoots and leaves are also edible; especially in the northern region of Nigeria. The matured seeds contain about 20% of edible oil [12, 13].

1.5. Kinetic Modelling

1.5.1. Kinetic Modeling of Vitamin C Loss in Leafy Vegetables

Time and temperature related changes in food quality could be modeled with the concept of chemical kinetics. The kinetic parameter like rate constant and activation energy provide useful information of the quality changes, that is, the change in concentration of vitamin C. the change is an index of concentration monitored under isothermal blanching conditions and is modeled with the differential equation (General rate equation):-

$$\frac{d[Vc]}{dt} = -k[Vc]^n \tag{1}$$

Where, V_c = concentration of vitamin C.

t = time

k = reaction rate constant

n = reaction order (a curve fitting parameter).

The quality response of most foods has been observed to follow either a zero order (n =0) or a first order (n = 1) reaction model [14, 18, 19]

1.5.2. Integral Method of Data Analysis

The integral method of analysis always puts a puts a particular rate equation to the test by integrating and comparing the predicted C versus t curve with experimental C versus t data. If the fit is unsatisfactory, another rate equation is guessed and tested. It should be noted that the integral method is especially useful for fitting simple reaction type's corresponding to elementary reaction testing the first – order rate equation of the following type [20].

$$-rA = -\frac{dVc}{dt} = k[Vc] \tag{2}$$

Rearranging equation (2)

$$-\frac{dVc}{Vc} = kdt \tag{3}$$

Integrating equation (3) we obtain

$$-\ln\frac{Vc}{Vc_O} = kt \tag{4}$$

2. Materials and Methods

2.1. Samples Collection and Preparation

The vegetables (*Moringa oleifera, hibiscus sabdarifa and hibiscus esculentus*) were collected fresh from the farm (lambun Bayara in Bauchi, Nigeria), in sealed and non-transparent polythene bags. The samples were rinsed under running water to remove any dirt and unwanted materials and then allowed to drain for about 5 minutes. 10g each was then weighed out for subsequent ascorbic acid determination. Convenient sampling method was used to collect the samples.

2.2. Preparation of Solutions

i) 5% Metaphosphoric Acid Solution:

5% metaphosphoric acid solution was prepared by dissolving 50g of metaphosphoric acid in 1 liter of distilled water (5g/100ml).

ii) 2,6-Dichlorophenol-Indophenol Solution:

2,6-Dichlorophenol-indophenol solution was prepared by dissolving 0.8g of 2,6-dichlorophenol-indophenol (DCIP) in 1liter of deionized water (0.8g/1000ml).

iii) Standard Ascorbic Acid Solution:

Standard ascorbic acid solution was prepared by dissolving 0.2g of standard ascorbic acid in 50ml of distilled water. The solution was covered with aluminum foil and refrigerated.

2.3. Standardizing the Dye (DCIP)

Standardizing the dye (DCIP) was accomplished by titrating the dye (DCIP) solution into a solution containing 1.0ml of standard ascorbic acid solution (4.0mg/1ml) and 9.0ml of 5%metaphosphoric acid solution. The end point of the titration was defined as pink color that persist through at least 15 seconds of swirling. The amount of ascorbic acid equivalent to 1.0ml of the dye was then calculated using equation (5).

$$\frac{ascorbic\,acid\,(mg)}{1.0ml\,of\,dye} = \frac{4.0mg\,of\,ascorbic\,acid}{dye\,titrated\,(ml)} \tag{5}$$

2.4. Measurement of Ascorbic Acid Concentration

10g of fresh vegetables was crushed in a mortar with a minimum volume of 5% metaphosphoric acid solution to form a fine paste. The paste was transferred into a beaker and the mortar and pestle rinsed several times with the 5% metaphosphoric acid solution, into the beaker. The mixture was thoroughly stirred and then filtered through Miracloth into a 100ml beaker and the filtrate made up to a mark with more 5%metaphosphoric acid solution. Ascorbic acid extract (10ml) was pipetted into a 100ml conical flask. The extract was titrated against the dye (DCIP) until a colorless end point is reached. For accuracy and precision, this procedure was repeated three times for each sample and blanching time.

2.5. Blanching

The steam blancher was switch-on, set at blanching temperature of 95°C and allowed to generate steam. 50g of fresh vegetables were weighed and placed on the blancher tray. The blancher was closed for one minute, after one minute the tray was removed and the vegetable transferred into a non-transparent polythene bag. The procedure above was repeated for 2, 3, 4, 5 and 6 minutes respectively. After blanching, same procedure as in 2.4 was repeated to measure the vitamin C contents of the vegetables and the result obtained was recorded in Tables 2 - 4.

3. Results and Discussion

3.1. Moringa Oleifera

Table 2 presents the change in concentration of ascorbic acid in Moringa *oleifera* leaves during blanching. The fresh sample before blanching had ascorbic acid concentration of 221mg/100g of sample. The concentration reduced by more than half after six minutes of blanching which is equivalent to 64.2% loss of ascorbic acid content. The result of the degradation and % loss during blanching is presented on Figure 1 and 2 respectively.

Table 2. *Vitamin C degradation in Moringa oleifera leaves during blanching at 95°C.*

Blanching time (min)	Conc. Of vitamin C (mg/100g)	% loss of vitamin C (%)	$-\ln\frac{Vc}{Vc_o}$
0	221.0	0	0
1	182.8	17.3	0.19
2	159.8	27.7	0.32
3	135.4	38.7	0.49
4	117.4	46.9	0.63
5	88.6	59.9	0.91
6	79.2	64.2	1.03

3.2. Hibiscus Sabdarifa

The result of effect of blanching on concentration of ascorbic acid in *hibiscus sabdarifa* (Roselle) leaves is presented on table 3. The fresh leaves before blanching was found to contain ascorbic acid concentration of 310mg/100g of the sample. After six minutes of blanching, the ascorbic acid concentration reduces to 142mg/100g which is equivalent to 54.2% loss. This result is presented on Figures 1 & 2 respectively.

Table 3. *Vitamin C degradation in hibiscus sabdarifa leaves during blanching at 95°C.*

Blanching time (min)	Conc. Of vitamin C (mg/100g)	% loss of vitamin C (%)	$-\ln\frac{Vc}{Vc_o}$
0	310.0	0	0
1	272.0	12.3	0.13
2	257.0	16.9	0.19
3	234.0	24.5	0.28
4	201.0	35.2	0.43
5	164.0	47.1	0.64
6	142.0	54.2	0.78

3.3. Hibiscus Esculentus

The changes in ascorbic acid concentration in *hibiscus esculentus* (Okra leaves) during blanching is presented in Table 4. The fresh leaves before blanching had ascorbic acid concentration of 194.3mg/100g of the sample. 69.7% loss of ascorbic acid content of the sample was recorded after blanching for six minutes. The result of the degradation and % loss during blanching is presented on Figure 1 and 2 respectively.

Table 4. *Vitamin c degradation in hibiscus esculentus leaves during blanching.*

Blanching time (min)	Conc. Of vitamin C (mg/100g)	% lost of vitamin C (%)	$-\ln\frac{Vc}{Vc_o}$
0	194.3	0	0
1	165.7	15.2	0.16
2	138.0	29.0	0.34
3	121.3	37.6	0.57
4	98.3	49.4	0.68
5	82.8	57.4	0.85
6	58.8	69.7	1.20

Figure 1 and 2 presents some plots of how blanching time affect the vitamin C concentration of leafy vegetables. Despite their different initial concentration of the vitamins, all the three vegetables follow the same pattern in all the three leafy samples at fixed temperature and pressure.

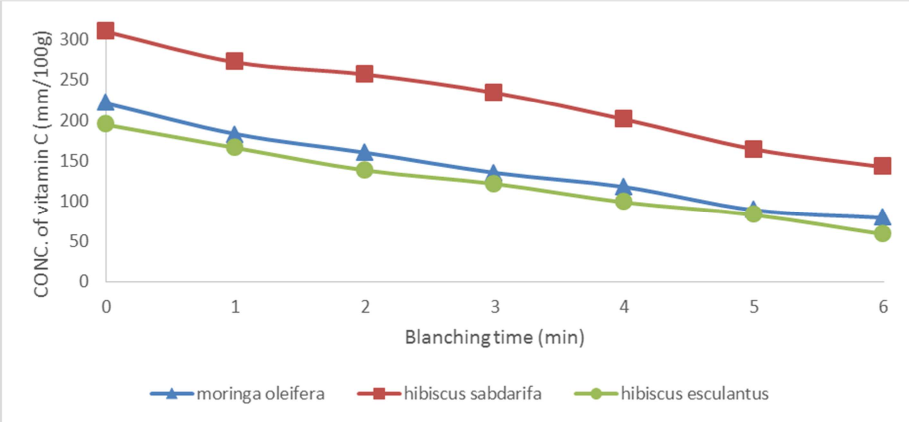

Figure 1. *Effect of blanching on vitamin C content of the three Leafy vegetables.*

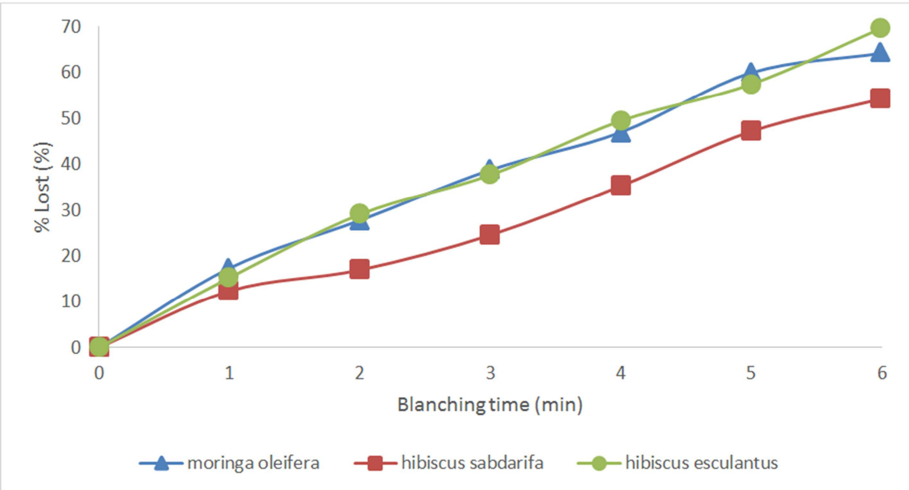

Figure 2. *% Loss of vitamin C concentration in the three leafy vegetables during Blanching.*

3.4. Kinetic Model Parameters Determination

Plots of $-ln\frac{V_c}{V_{co}}$ against time (t) are presented in figures 3, 4 and 5 for the three leafy vegetables. The reaction rate constants for the degradation of the three vegetable samples were obtained by integral fitting of the kinetic model equation in which the slope of the plot of $-ln\frac{V_c}{V_{co}}\ vs\ t$ is the reaction rate constant k. The order of the reaction n, was found to be first order for all the three vegetables since the plot gives a straight line from the origin which indicates first order from integral method of analysis, this result is in agreement with the results obtain in modeling other food quality parameters by other researchers who concludes that "For qualities changes in biological systems such as foods or drugs, the reaction order n has generally been shown to be either 0 or 1, depending on the reaction involved" [14,17-19].

From figure 3, the reaction rate constant k for *moringa oleifera* was found to be 0.1681 (min^{-1}). The reaction rate constant (k) for *hibiscus sabdarifa* was found to be 0.1216 (min^{-1}) as indicated on figure 4 below. From figure 5, the reaction rate constant for hibiscus esculentus was found to be 0.1881 (min^{-1}). The kinetic models for all the three leafy vegetables fits into a first order kinetics. The model equation was solved using integral method of analysis.

Since from the integrated rate expression,

$$-ln\frac{V_c}{V_{co}} = kt \tag{6}$$

$$V_{c(t)} = V_{co}\exp(-kt) \tag{7}$$

Where;

V_c = the concentration of vitamin C at time t.

V_{co} = the initial concentration of vitamin C in the vegetable before blanching

k = the kinetic rate constant.

Since V_{co} and k for all the three vegetables are known, equation (7) can be used to predict Vc in any of the three vegetables for any blanching time (t).

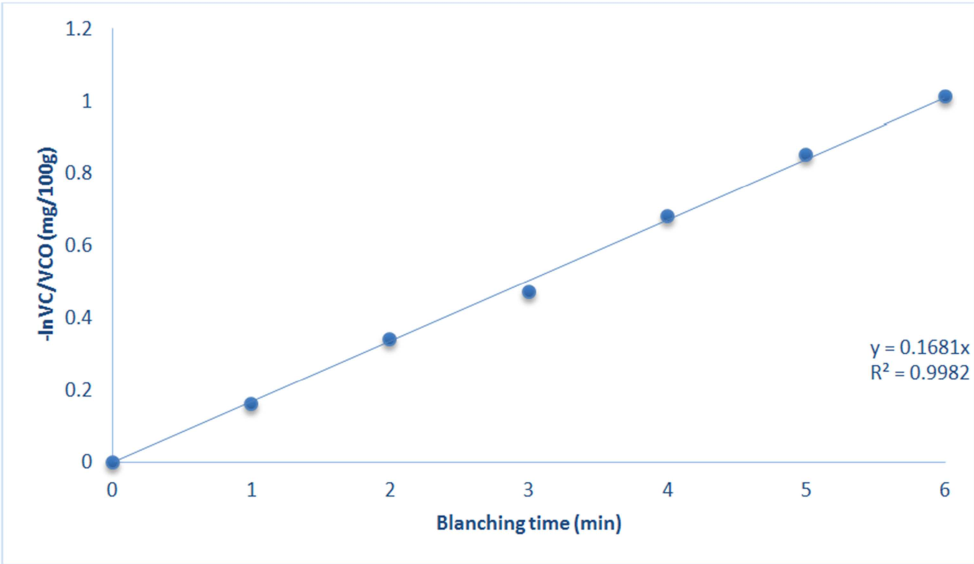

Figure 3. *integral fitting of vitamin C degradation in moringa oleifera leaves during blanching.*

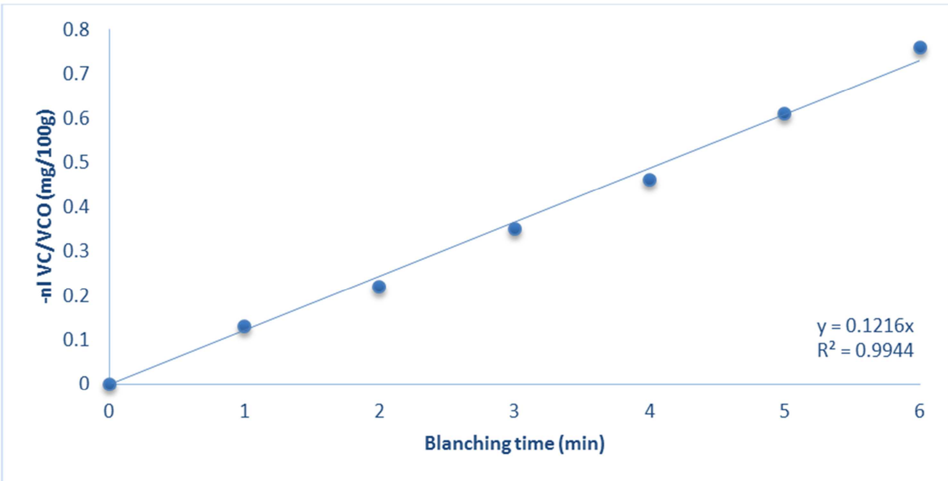

Figure 4. *integral fitting of vitamin C degradation in hibiscus sabdarifa leaves during blanching.*

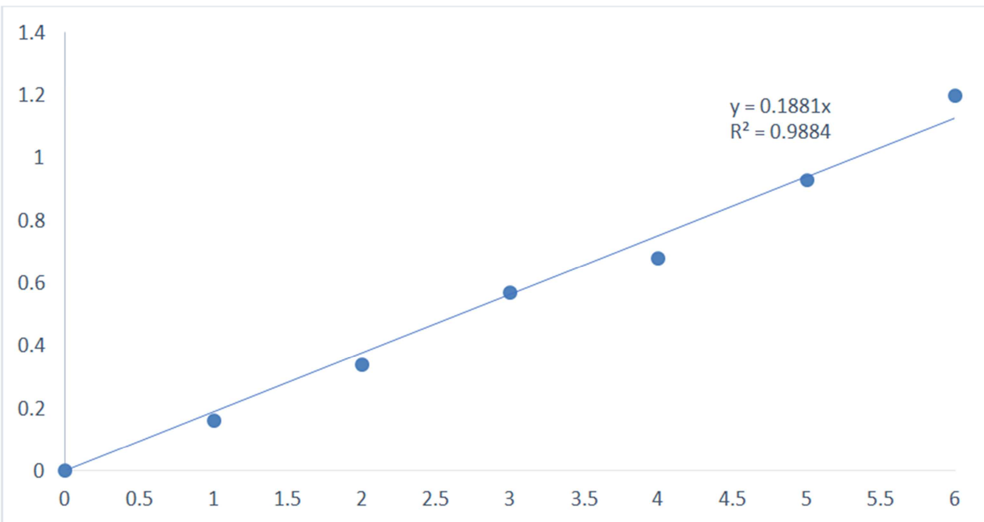

Figure 5. *integral fitting of vitamin C degradation in hibiscus esculentus leaves during blanching.*

4. Conclusion

The main aspect of this study were the establishment of reliable kinetic models of vitamin C degradation for *Moringa oleifera, hibiscus sabdarifa and hibiscus esculentus* during blanching at deferent blanching times. The result of this study indicates that the quantity or concentration of vitamin C in *Moringa oleifera, hibiscus sabdarifa and hibiscus esculentus* was strongly affected by the blanching. Although the three vegetables have different initial concentration of vitamin C prior to blanching, the rate of degradation with blanching time is similar. The three vegetables were blanched for 1 to 6 minutes and the effect of blanching on vitamin C content of all the three vegetables was significant. This studies also indicate that the rate of vitamin C degradation decreases with increase in blanching time. Kinetic equations for the degradation of three leafy vegetables developed was restricted to blanching conditions in this work.

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