Original Research Article
Kinetics of Chlorophyll Degradation in Malabar Spinach (*Basellae alba)* and Waterleaf (*Talinum triangulare*) during Hydrothermal Processing

ABSTRACT

Hydrothermal processing of vegetables often results in a number of changes including degradation of chlorophyll, the green plant pigment that is considered to be of therapeutic benefits. Freshly harvested leafy vegetables Malabar spinach (*Basella alba*) and waterleaf (*Talinum triangulare*) were obtained and subjected to various hydrothermal conditions (60, 65, 70, 75 and 80˚C at 10, 15 and 20 minutes) simulating typical cooking conditions. Changes in chlorophyll was determined using spectrophotometric analysis and the kinetics of degradation for each of the vegetables were determined at varying temperatures and times. Kinetics analysis revealed that the degradation of chlorophyll in Malabar spinach and waterleaf followed the first order reaction. The rate constant obtained from the kinetic analysis provided insights into the reaction rates and allowed for the prediction of chlorophyll degradation during hydrothermal processing. For Malabar spinach, blanching at 65˚C for 5 minutes and waterleaf at 60˚C for 5 minutes resulted in the lowest percentage decreases in chlorophyll concentration during processing. Blanching temperature and time have significant effects on degradation of chlorophyll of Malabar spinach and waterleaf which could consequently determine the economic value and overall degree of acceptability of the green leafy vegetables.

Keywords:Malabar spinach, waterleaf, kinetics, chlorophyll degradation, hydrothermal processing

1. INTRODUCTION

Consumption of vegetables in many regions of the world is on the increase because they are often associated with a number of health benefits. Vegetables are highly consumed because they are good sources of vitamins, essential mineral elements and dietary fibre [1,2,3]. Addition of vegetable to daily food intake helps to boost mental health, reduce incidences of cancer and life threatening diseases [3,4,5].

Green plants have the ability to make their own food. They do this through a process called photosynthesis which uses a green pigment called chlorophyll. A pigment is a molecule that has a particular color and can absorb light at different wavelengths. Naturally occurring pigments in plants are many and varied. Chlorophyll is the pigment that gives plants their green colour. Chlorophyll is found in the thylakoids membrane of the chloroplast, a specialized structure naturally occurring in photosynthetic plants. Natural pigments, including chlorophyll, absorb and reflect various wavelengths of light. A light absorbing pigment, chlorophyll is called a photoreceptor. Chlorophyll a absorbs light in the blue-violet region and reflects green light while chlorophyll b absorbs red light and reflects the green

light. A chelating agent with a central magnesium (Mg) ion bonded to a larger organic molecule, chlorophyll a and b has the molecular formulae of $C_{55}H_{72}MGN_4O_5$ and $C_{55}H_{70}MGN_4O_{6,}$ respectively. Chlorophyll in plants is useful for many purposes. It is the pigment that enables plants to manufacture their own food during the process of photosynthesis. Chlorophyll enables plants to release oxygen into the environment during photosynthesis. Amount of chlorophyll in plants can be used to determine nutritional status [6]. The use of chlorophyll from plants as alternative therapies is not uncommon in many communities in developing countries; chlorophyll has been proven to have health benefits. Chlorophyll and its derivatives have been shown to increase red blood cells, have antioxidant properties and to reduce the effect of deleterious carcinogens [7]. Moreover, the economic value, marketability, and the overall degree of acceptability of many plants particularly leafy vegetables are dependent on the greenness of stems and leaves.
Malabar spinach (*Basellae alba)* and waterleaf (*Talinum triangulare*) are two green leafy vegetables grown in the southern part of Nigeria and some tropical regions of the world. Malabar spinach and waterleaf are locally called *amunututu* and *gbure*, respectively. There are two main species of Malabar spinach, a specie with green stems and a specie with red-purple colour. Although a native of India, Malabar spinach is grown in other tropical regions of the world. Waterleaf, a short life-span plant that can adapt to varying climatic conditions is quite resistant to pests and diseases [8,9,10,11]. The two leafy vegetables are rich in vitamins A, B and C, as well as iron and calcium. They are low in calorie but high in antioxidants and rich sources of fiber [1,12,13,14]. The leaves have mucilaginous texture. They are often used as soup thickeners in soups and stews in African and Asian cuisines [13,15,16,17]. Waterleaf has been used as remediation of soil exposed to heavy metals [18].
Blanching of vegetables inactivates enzymes responsible for senescence and induces structural and chemical changes in the tissue which initiate chlorophyll degradation. Understanding degradation kinetics, including reaction order, rate constant and activation energy, is essential to predict loss of quality during thermal processing and storage [19]. Although studies have been carried out on changes that occur in chlorophyll content during processing, there is dearth of information on kinetic degradation of chlorophyll in Malabar spinach (*Basellae alba)* and waterleaf (*Talinum triangulare*). Hence, the study is aimed at examining the kinetic degradation of chlorophyll in Malabar spinach (*Basellae alba)* and waterleaf (*Talinum triangulare*) during hydrothermal processing.

2. MATERIAL AND METHODS

2.1 Material

Freshly harvested Malabar spinach – green stem (*Basellae alba*) locally called *amunututu* – Figure 1a and waterleaf (*Talinum triangulare*) locally known as *gbure* – Figure 1b were obtained from a local farm in Ogbomoso (8˚08'N, 4˚15'E) and brought to Teaching and Research Unit of Agronomy Department, Ladoke Akintola University of Technology, Ogbomoso for identification

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Figure 1a: Malabar spinach (*Basella alba*) Figure 1b: Water leave (*Talinum triangulare*)

2.2 Sample preparation and hydrothermal processing

The leaves were plucked, washed with clean cold water and then placed on a stainless steel perforated container to allow proper draining of the water from the leaves. The washed vegetables was blanched in a thermo regulated water bath (Model HH-W21-Cr42II, Techmel and Techmel, Texas, USA),at the temperature of 60, 65, 70, 75, 80, 85 and 90 ˚C and at 0, 5 10, 15, 20, 25 and 30 minutes for each temperature. Each of the processed samples was analysed for its chlorophyll content using the method described below.

2.3 Determination of chlorophyll content

Chlorophyll content was determined immediately after blanching by weighing 0.2 g of the leaf and placed in a test tube. 10 cm^3 of 80% acetone was added to the leaf inside the test tube and left for 30 min. About 3 cm^3 of the resulting green extract was poured into a 4 ml cuvette and then placed in a spectrophotometer --- UVV is -NIR V-600, JASCO spectrophotometer. The absorbance of the extract was measured at 645 nm for chlorophyll a and at 663 nm for chlorophyll b. The chlorophyll pigment concentration was calculated using Lichtenthaler's equation []20].

$$Chla = \frac{1395 \text{ x } A665 - 6{,}88 \text{ x } A649}{d \text{ x } W \text{ x } 1000} \text{ x } V \text{ x } D \left(\frac{mg}{g}\right) \quad (1)$$

$$Chlb = \frac{24{,}96 \text{ x } A649 - 7{,}32 \text{ x } A665}{d \text{ x } W \text{ x } 1000} \text{ x } V \text{ x } D \left(\frac{mg}{g}\right) \quad (2)$$

$$Total..Chl = \frac{6263 \text{ x } A665 + 18208 \text{ x } A649}{d \text{ x } W \text{ x } 1000} \text{ x } V \text{ x } D \left(\frac{mg}{g}\right) \quad (3)$$

where: A= absorbance atspecified wavelength, V = volume of total extract (ml), W = fresh weight (g), D = coefficient of dilution, d = thickness of cuvette (mm), 1000 = conversion factor µg– mg

2.4 Degradation kinetics of chlorophyll determined

Kinetic model for chlorophyll degradation of Malabar spinach and waterleaf was obtained using a dynamic test approach [21]. An empirical first order kinetic model was used:

$$-\frac{dc}{dt} = kc \quad (4)$$

where, c is the concentration of chlorophyll(normalize with respect to initial concentration). The first order rate constant (k) was Arrhenius temperature dependent.

$$ln\ \frac{C}{C_0} = -kt \quad (5)$$

where, C is the concentration of the chlorophyll a , b, at any time t, C_0 is the initial chlorophyll content, k is the first-order rate constant (min^{-1}), t: time (min).

2.5 Half-life

Half-life which is the time taken to disintegrate the chlorophyll content present in vegetable leaf to half of its original value was determined by using rate constant (k) as

$$\text{Half-life} = {}^{0.693}/_{k} \quad (6)$$

3. RESULTS AND DISCUSSION

3.1 Kinetics of Chlorophyll Degradation in Waterleaf during Hydrothermal Processing

The chlorophyll content of *Talinum triangulare* (waterleaf) during blanching at different temperatures for varying length of time is presented in Table 1. The raw sample (control) of waterleaf contained 3.76 mg/l of chlorophyll. Processing by blanching at 60 ˚C for 5 min did not cause any significant change in the chlorophyll content. Blanching of waterleaf at 60 C for 10 min cause the chlorophyll content to decrease to 3.46 mg/l and there was further

decrease to 3.30 mg/l after blanching for 15 min. The chlorophyll content of waterleaf was reduced to 3.07 mg/l after blanching for 20 minutes at 60 ˚C. At 65 ˚C, the chlorophyll content of waterleaf was 3.76, 3.50, 3.36, 3.26 and 3.10 mg/l for blanching time of 0, 5, 10, 15 and 20 min, respectively. There was significant difference (P =.05) in the chlorophyll content of waterleaf when it was processed at 70 ˚C for varying time of blanching. The chlorophyll content of the blanched samples at 70 ˚C was 3.76, 3.15, 3.11 3.03 and 3.01 mg/l at the blanching time of 0, 5, 10, 15 and 20 min, respectively. Concentration of chlorophyll in the raw sample decreased from 3.76 to 2.50 mg/l after blanching at 75 ˚C. The chlorophyll content for samples blanched at 80 ˚C were 3.76, 3.03, 2.74, 2.38 and 2.15 mg/l at blanching time of 0, 5, 10, 15 and 20 min, respectively. Generally, there was a reduction in the concentration of chlorophyll in the leaf with increase in temperature and processing time. The percentage reduction increased with increase in processing temperature and time. Thus, the highest percentage reduction of 42.82 % was recorded at 80 ˚C. A similar result was obtained on ascorbic acid and chlorophyll retention of leafy vegetables during processing [22].

Table 1. Changes in chlorophyll content of *Talinum triangulare* (waterleaf) during hydrothermal processing at varying temperature and times (mg/l)

Time (min)	60 ˚C	65 ˚C	70 ˚C	75 ˚C	80 ˚C
0	3.76 ± 0.00^{d} {0.00}	3.76 ± 0.00^{d} {0.00}	3.76 ± 0.00^{d} {0.00}	3.76 ± 0.00^{d} {0.00}	3.76 ± 0.00^{d} {0.00}
5	3.76 ± 0.00^{d}	3.50 ± 0.02^{d} {0.00}	3.15 ± 0.08^{b} {7.42}	3.07 ± 0.02^{c} {16.22}	3.03 ± 0.42^{d} {18.35} {19.41}
10	3.46 ± 0.05^{c}	3.36 ± 0.06^{c} {7.97}	3.11 ± 0.00^{ab} {10.63}	3.00 ± 0.00^{c} {17.28} {20.21}	2.74 ± 0.09^{c} {27.12}
15	3.30 ± 0.03^{b}	3.26 ± 0.06^{b} {12.23}	3.03 ± 0.04^{a} {13.29} {19.41}	2.78 ± 0.11^{b} {26.06}	2.38 ± 0.03^{b} {36.70}
20	3.07 ± 0.10^{a}	3.10 ± 0.02^{a} {18.35}	3.01 ± 0.01^{a} {17.55} {19.95}	2.50 ± 0.11^{a} {33.51}	2.15 ± 0.08^{a} {42.82}

Values above are means of duplicate ± the standard deviation
Means with different letters along the same column are significantly different at (P = .05)
Values in parenthesis represent percentage decrease in chlorophyll content during processing

3.2 Kinetics of Chlorophyll Degradation in Malabar Spinach during Hydrothermal Processing

The concentration of chlorophyll in the leaf of Malabar spinach (*Basella alba*) during hydrothermal processing at varying time is presented in Table 2. The raw sample of *Amunututu*contained 4.15 mg/l of chlorophyll. At 60 ˚C, the concentrations of chlorophyll were 4.23, 4.21, 4.09 and 4.08 mg/l for blanching time of 5, 10, 15 and 20 min, respectively. At 65 ˚C, the chlorophyll content ranged from 3.76 mg/l for the raw sample to 3.10 mg/l for the sample blanched for 20 min. Similarly, at temperature of 70 ˚C, the chlorophyll content were 4.15, 4.00, 4.00, 3.78 and 3.54 at blanching time of 0, 5, 10, 15, and 20 min, respectively. The concentration of chlorophyll ranged from 4.15 mg/l to 3.03 mg/l when the leaf was processed by blanching at 75 ˚C, the highest percentage reduction of 45.78% was recorded when it was processed at 80 ˚C. Thus, at varying temperatures and times, there was significant reduction (P = .05) in the concentration of chlorophyll during hydrothermal

processing. As in the case of *Talinum triangulare*, the chlorophyll content decrease with increase in processing time and temperature.

In general, there was significant difference in the chlorophyll content ((P = .05)among the samples processed at different temperatures and blanching times. Initially, there was a rapid decrease in chlorophyll content, followed by a slower degradation phase. The degradation rates were influenced by both time and temperature. Higher temperatures accelerated chlorophyll degradation, leading to faster color loss. Also, longer processing times also contributed to increased chlorophyll degradation. Thus, there was a decrease in chlorophyll content of the samples with increase in blanching time at varying blanching temperatures. Similar finding was reported during blanching of bronccoli florets [22]. Chlorophyll is sensitive to heat treatment. The loss of the green color during heat treatment is mainly attributed to the transformation of chlorophyll -- of green color, to pheophytins -- of brown color, due to the replacement of the magnesium ion in the porphyrin ring of the chlorophyll by two ions of hydrogen, reaction which takes place in acid environment [23,24]. The results obtained in this study are comparable to earlier findings on thermal degradation of kinetic of chlorophyll of pureed coriander leaves [24], chlorophyll retention and drying characteristic of ivy ground leaf [25], thermodynamic and kinetics study of phenolic degradation and colour of yacon [26], degradation kinetic of pumpkin [27] and degradation of *amaranthuscaudatus* [24].

Table 2. Changes in chlorophyll content of Malabar spinach (*Basellae alba*) during hydrothermal processing at varying temperatures and times (mg/l)

Time (Min)	60 ˚C	65 ˚C	70 ˚C	75 ˚C	80 ˚C
0	4.15 ± 0.12^{ab} {0.00}	4.15 ± 0.12^{ab} {0.00}	4.15 ± 0.12^{ab} {0.00}	4.15 ± 0.12^{ab} {0.00}	4.15 ± 0.12^{ab} {0.00}
5	4.15 ± 0.00^{ab} {0.00} {0.00}	4.15 ± 0.08^{a} {3.61}	4.00 ± 0.00^{c} {9.87}	3.74 ± 0.06^{c} {17.34}	3.43 ± 0.11^{c}
10	4.08 ± 0.01^{a} {1.69} {2.89}	4.03 ± 0.03^{a} {3.61}	4.00 ± 0.00^{c} {15.90}	3.49 ± 0.04^{b} {33.97}	2.74 ± 0.16^{b}
15	4.09 ± 0.01^{a} {1.44}	3.96 ± 0.06^{a} {4.57}	3.78 ± 0.35^{b} {8.92}	3.33 ± 0.14^{b} {19.76} {38.31}	2.56 ± 0.00^{ab}
20	4.08 ± 0.02^{a} {1.69}	3.90 ± 0.01^{a} {3.61}	3.54 ± 0.08^{a} {14.69} {26.98}	3.03 ± 0.08^{a} {45.78}	2.25 ± 0.17^{a}

Values above are means of duplicate plus the standard deviation
Different letters along the same column are significantly different at (P = .05)
Values in parenthesis represent percentage decrease in chlorophyll content during processing

3.3 Determination of kinetic degradation parameter

In order to arrive at the reaction rate constants for the degradation kinetics of *Basella alba* and *Talinum triangulare*, a first order degradation was presumed. Table 3 and 4 shows degradation kinetic of *Basellae alba* (Malabar spinach)and *Talinum triangulare* (waterleaf), respectively. The plot of $\mathrm{Ln}\left(\frac{C_t}{C_0}\right)$ against time in which rate constant K was obtained as slope of the graph. From the graph (Figure 1a and 1b), correlation coefficient obtained was greater than 0.9 in all blanching temperature (60, 65, 70, 75 and 80 ˚C) and time (5, 10, 15 and 20 min) which indicated that the degradation followed the first order kinetics. The values of rate constants for the degradation of *Basella alba* and *Talinum triangulare*are presented in Table 5 and Table 6, respectively. The rate constant increase from 0.005 – 0.054 min^{-1} and 0.0180

– 0.1150 min^{-1} for *Basella alba* and *Talinum triangulare,* respectively. It was observed that the rate of chlorophyll degradation of *Basella alba* and *Talinum triangulare*increased with increase in temperature and blanching time. This finding was in agreement with an earlier finding where the rate constant for kinetic degradation of ODAP in *Lathyrus sativus* increased with increase in temperature [28]. Activation energy Ea ($calm^{-1}$) was calculated as a product of gas constant, R (1.987 $calM-^{1}K^{-1}$) and the slope of the graph obtained by plotting Lnk versus '1/T'. Figure 3 (a and b). The gradient of these graph gave the activation energy and is given as 28.996 KJ and 10.214 KJ

Table 3. Kinetics of chlorophyll degradation of *Basella alba* at different blanching temperatures and times.

	$Ln\left(\frac{C_t}{C_0}\right)$				
Time (min)	60 ˚C	65 ˚C	70 ˚C	75 ˚C	80 ˚C
5	0.019	0	-0.037	-0.04	-0.190
10	0.0144	-0.029	-0.037	-0.173	-0.415
15	-0.0145	-0.047	-0.101	-0.220	-0.483
20	-0.0170	-0.036	-0.159	-0.315	-0.612

Table 4. Kinetics of chlorophyll degradation of *Talinum triangulare* at different blanching temperatures and times.

	$Ln\left(\frac{C_t}{C_0}\right)$				
Time (min)	60 ˚C	65 ˚C	70 ˚C	75 ˚C	80 ˚C
5	0	-0.071	-0.177	-0.215	-0.215
10	-0.083	-0.112	-0.180	-0.225	-0.309
15	-0.130	-0.142	-0.215	-0.301	-0.457
20	-0.202	-0.193	-0.222	-0.408	-0.558

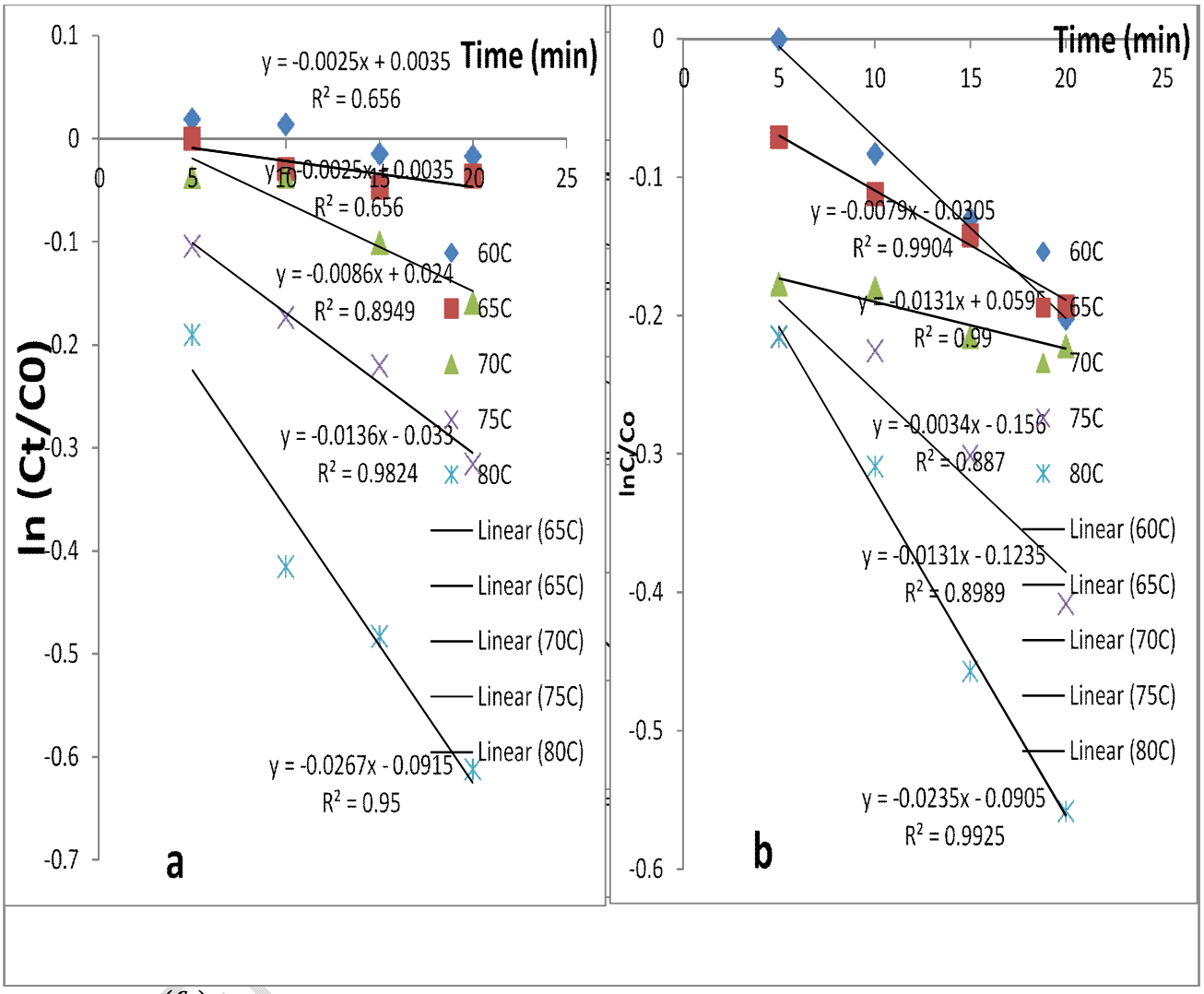


Fig 2. Plot of Ln$\left(\frac{C_t}{C_0}\right)$ against time for (a*) Basella alba*and (b) *Talinum triangulare*

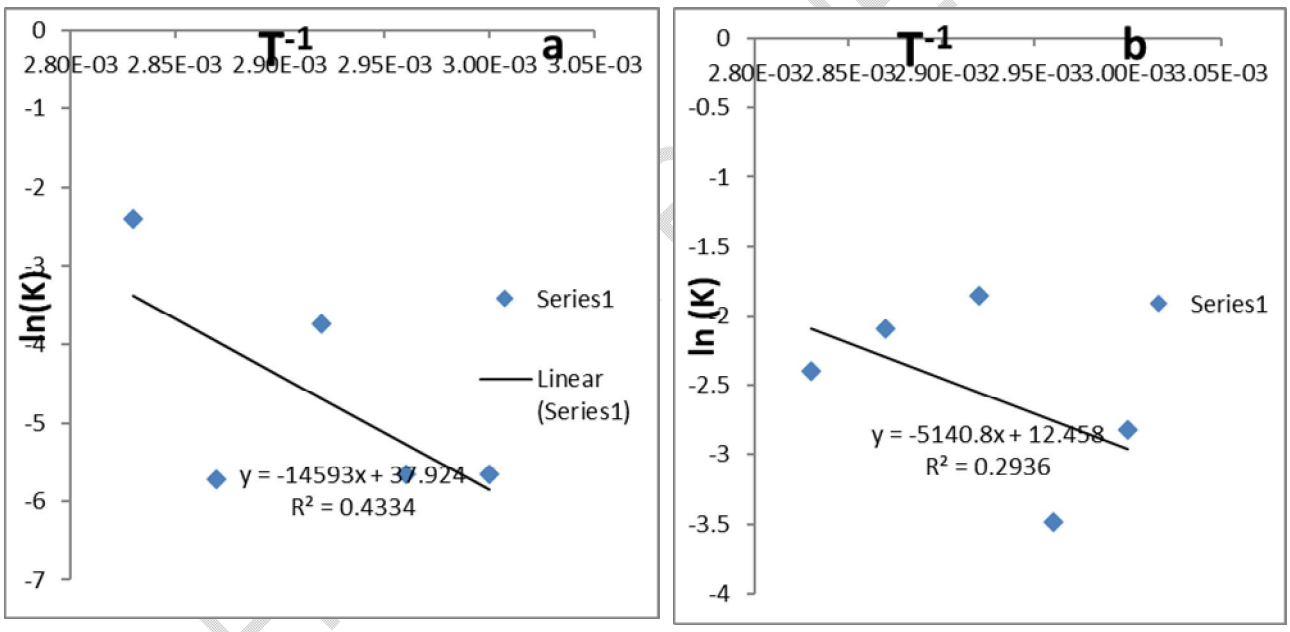
Table 5. Rate constant for *Basella alba*

Temperature ˚C	K	Ln(K)	1/T
60	0.0185	-3.989	3.0E-3
65	0.054	-2.919	2.96E-3
70	0.0115	-4.465	2.92E-3
75	0.005	-5.298	2.87E-3
80	0.0115	-4.465	2.83E-3

Table 6. Rate constant for *Talinum triangulare*

Temperature ˚C	K	Ln(K)	1/T
60	0.0595	-2.8218	3.0E-3
65	0.0305	-3.490	2.96E-3
70	0.156	-1.858	2.92E-3
75	0.1235	-2.0915	2.87E-3
80	0.0905	-2.4024	2.83E-3

Figure 4. Plot of ln (K) against $T^{-1}$for (a) *Basella alba*and (b) *Talinum triangulare*



4. CONCLUSION

This study has shown that chlorophyll degradation occurs rapidly during hydrothermal processing leading to the loss of characteristic green color of the leaves. The degradation of chlorophyll in Malabar spinach (*Basella alba*)and waterleaf (*Talinum triangulare*) follows first-order kinetics, with the rate of degradation depending on temperature and blanching time. Higher temperatures and longer processing times result in increased chlorophyll degradation. Hence, understanding the kinetics of chlorophyll degradation in Malabar spinach and waterleaf during hydrothermal processing is expedient for optimizing processing conditions to preserve the colour and other quality characteristics of these leafy vegetables which are considered to be sources of alternative therapies.

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