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KINETIC OF CHLOROPHYLL DEGRADATION AND COLOR CHANGE IN BITTER GOURD DURING HEAT TREATMENT

Duong Thi Phuong Lien*

*Food Technology Department, College of Agriculture and Applied Biology, Cantho University, Vietnam

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ABSTRACT

Thermal degradation kinetics of chlorophylls and visual green color loss in bitter gourd were investigated at 70, 80, 90 and 100°C. The loss of visual green color, as represented by the change of the –a value and the –a/b ratio measured by tristimulus colorimeter, All the degradation of chlorophyll a, chlorophyll b as well as visual green color loss followed a first–order reaction. The rate constants of all parameters increased with increasing temperature and followed the Arrhenius model. The activation energies for chlorophylls a and b were determined as 32.90 ± 2.68 and 49.96 ± 3.86 kJ/mol, while those for –a value and –a/b ratio were found as 22.75 ± 1.96 and 53.55 ± 4.08 kJ/mol, respectively.

KEYWORDS: Chlorophyll, bitter gourd, kinetics, color, activation energy.

INTRODUCTION

Bitter gourd *(Momordica charantia L.)* belong to the family Cucurbitaceae [1] and is one of the most popular vegetables in many Asian countries and. Normally, it is prepared as cooked meal that is a delicacy for the East and Southeast Asian people [2].

However, thermal processing induces structural and chemical variations to the tissue of vegetables that often result in color changes [3, 4]. Prolonged cooking of green vegetables such as bitter gourd has an olive green color compared to the bright green color of raw materials. The reason for the color changes during thermal processing green vegetables mainly due to the conversion into pheophytins from chlorophylls [5] that are the most widely distributed plant pigments responsible for the characteristic green color of fruit and vegetables [6]. Formation of pheophytins during heating due to the replace of two hydrogen ions in acidic medium into magnesium site in the chlorophyll ring result in the convert from green chlorophylls to the olive brown pheophytins [7, 8].

The major chlorophylls in foods are chlorophyll a and chlorophyll b. The molecular structure of chlorophyll a has been established by total synthesis of the tetrapyrrole moiety [9] and the C-20 terpenoid alcohol, phytol [10]. While chlorophyll b is distinguished from chlorophyll a by a 7-formyl instead of the 7-methyl-substitutent [11]. The ratio of chlorophyll a and b occur in fruits and vegetables approximate of 3:1 [12]. Chlorophylls a and b are typically found in higher plants and occur in approximate ratio of 3:1 in fruits and vegetables. They are also different in case of color where chlorophyll a appears blue-green and chlorophyll b possesses yellow-green [13], as well as their thermal stability. Chlorophyll a was reported to be thermally less stable than chlorophyll b [3, 14].

Chlorophylls are known to be easily degraded by conditions such as dilute acids, heat, light and oxygen [15]. Since color is a major sensory characteristic in determining product acceptability, it is important to prevent or at least minimize chlorophyll degradation during thermal processing in food industry. The researches on determining the discoloration of vegetables during processing are mainly based upon quantitative analyzes of chlorophyll pigments. On the other hand, some investigators used colorimetric parameters to determine kinetic parameters for the color changes in vegetables. Therefore, the kinetic parameters including reaction order, rate constant and activation energy are essential to predict the quality loss during thermal processing [12]. Various kinetic models are used to describe the color changes in fruits and vegetables during thermal treatment and storage, such as green peas [12], green asparagus [16], chestnut kernel [17], apple [18], jackfruit [19], hazelnuts [20] and broccoli [21]. According to these authors, the kinetic models of color degradation follow first–order kinetic models.

The CIE–L, a, b system is a versatile and reliable method to assess the color of fruit and vegetables and its changes during storage and processing [22, 23]. The parameters a and b express the green–red and blue–yellow axis, respectively [23]. The –a value has been used as a physical parameter to represent greenness in color measurement [24, 21]. Beside, Cano and Marin (1992) [25] have monitored changes in chlorophyll content by the ratio of –a to b (–a/b value) for canned green peas and green beans.

The objectives of this study were to determine the degradation kinetics of chlorophyll a and chlorophyll b during blanching from 70 to 100ºC. In addition, the correlation of the chlorophyll a and chlorophyll b contents with the visual green color using CIE–L, a, b color system in thermally treated bitter gourd were analyzed.

MATERIALS AND METHODS

Materials and heat treatment

Bitter gourds *(Momordica charantia L.)* were supplied from Metro Super Market, Cantho, Vietnam. They were washed with tap water and drained. Cleaned bitter gourds were cut into 2cm thick rings for removing the seeds, and then were cut again into 2 × 2 cm^2 pieces. They were placed in distilled water baths set at 70°C, 80°C, 90°C and 100°C. Five pieces were removed at the time intervals (0, 1, 3, 5, 10, 15 and 20 min) and immediately cooled in running water at 20°C.

Determination of color values

The –a and b values for each sample were measured at least three points by a tristimulus colorimeter (Model CR–300, Minolta Chromameter, Japan). The data on the average of –a and the –a/b ratio values from measured points were also used to analyze.

Chlorophylls a and b determination

Chlorophylls a and chlorophyll b were analyzed and calculated as described by Askar and Treptow (1993) [26]. Samples were extracted by 85% acetone solution in a dark condition. The crude pigment is assayed after spectrophotometrically measurements the extracts at 644 and 662 nm. Chlorophyll a and chlorophyll b contents were calculated in the unit of mg/100g DW.

Kinetic models

Foods are generally complex biological systems. A number of reactions take place during thermal processing, either in series or in parallel, and competing. The final quality lossmay be the results of many interacting and complex reactions rather than a single elementary step [27]. In order to determine the color change of food materials as a function of heat treatment time, several equations for the application of color change kinetics have been published in the literature [28-30]. Generally, the rate of change of a quality factor C can be represented by equation (1).

$$\frac{dC}{dt} = -kC^n \qquad (1)$$

Where (k) is the kinetic rate constant (1/min), (C) is the concentration of a quality factor at time t, and (n) is the order of reaction. In this study, C displayed the chlorophyll a and chlorophyll b contents. For the evidences in majority of foods, the time dependence relationships appear to be described by first-order kinetic models. So, after integration the equation (1) can be written as equation (2):

$$C = C_0\exp(-kt) \qquad (2)$$

The dependence of the degradation rate constant (k) on temperature was quantified by the Arrhenius equation as equation (3):

$$k_t = A_0\exp\left(\frac{-E_a}{RT}\right) \qquad (3)$$

where A_0 is the preexponential factor (1/min); E_a is activation energy (J/mol); R is the gas constant (8.314 J/mol·K), and T is absolute temperature (K). The E_a value can be calculated from the slope of the experimentally developed k–T curve by Equation (3).
Kinetic modeling was carried out with the contents of chlorophyll a and chlorophyll b degradated as well as the green color loss during thermal processing.

Statistical analysis
The experimental results were expressed as mean ± standard deviation of three replicates and the linear regressions were established using the Microsoft Excel 2007.

RESULTS AND DISCUSSION

Thermal degradation of chlorophyll
Thermal degradation of chlorophylls a and b in bitter gourd was studied in the temperature range of 70–100°C with 10°C intervals. The degradation of chlorophylls a and b in bitter gourd followed a first–order reaction kinetic model as Equation (2), the plots of $\ln(\text{chlorophyll}_t/\text{chlorophyll}_0)$ varies to heating time were displayed in **Figures 1** and **Figure 2**. **Table 1** showed the reaction rate constants (k) and the determination coefficients (R^2) for the degradation of chlorophylls at all temperatures studied.

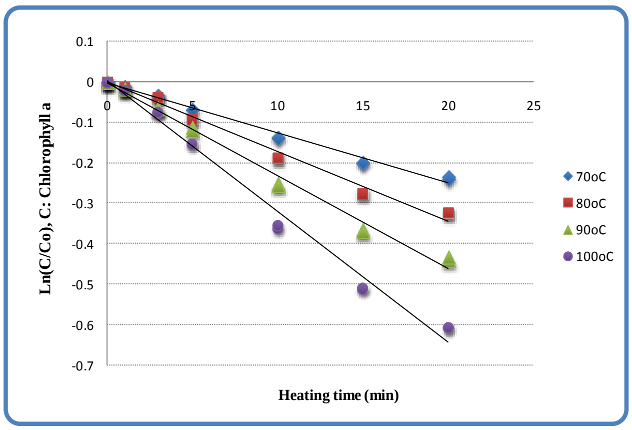

Figure 1: **Changes in chlorophyll a of bitter gourd during heating at various temperatures**

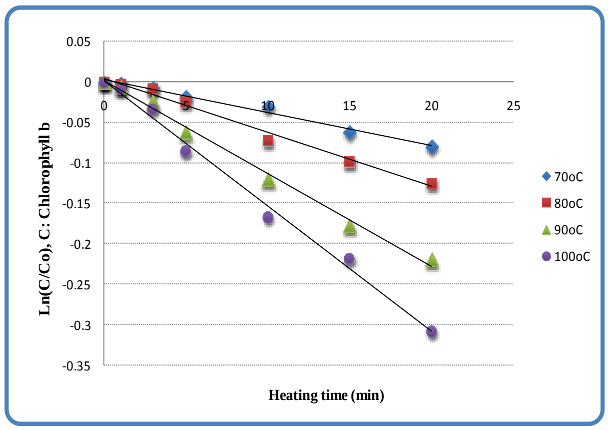

Figure 2: **Changes in chlorophyll b of bitter gourd during heating at various temperatures**

Table 1. Kinetic rate constants for chlorophyll a and b degradation during heat treatment of bitter gourd at different temperatures

Parameters	Heating temperature (°C)			
	70	80	90	100
Chlorophyll a degradation rate constant k_{Chla} (min^{-1})	$0.0126^a \pm 0.0006$	$0.0173^b \pm 0.0008$	$0.0231^c \pm 0.0008$	$0.0321^d \pm 0.0007$
Determination coefficients (R^2)	0.990	0.988	0.990	0.988
Chlorophyll a degradation rate constant k_{Chlb} (min^{-1})	$0.0039^a \pm 0.0002$	$0.0064^b \pm 0.0006$	$0.0114^c \pm 0.0007$	$0.0153^d \pm 0.0012$
Determination coefficients (R^2)	0.982	0.983	0.993	0.993

(Mean ± SD, The values showing different superscripts within a row are significantly different at $p < 0.05$)

Results from **Table 1** showed that the rate constant chlorophyll a degradation fasted significantly ($p < 0.05$) from 0.0126 ± 0.0006 to 0.0321 ± 0.0007 min^{-1} with increase in heating temperature. Similarly, the rate constant of

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degradation of chlorophyll b was also significant faster at high heating temperature and ranged from 0.0039 ± 0.0002 to 0.0153 ± 0.0012 min^{-1}. In addition, chlorophyll a degraded from 2.1 to 3.3 times faster than chlorophyll b depending on temperature, indicating that chlorophyll a is thermally less stable than chlorophyll b [3, 14].
This rule in chlorophylls degradation during heating process is similar to the result from study of Erge *et al.* (2008) which showed the increasing in the chlorophylls degradation constants with the increasing in heating temperature when heating green peas from 70 to 100°C [12]. They also found out the rate of chlorophyll a degradation was 12 to 18 times faster than chlorophyll b. Lajollo *et al.* (1971) [31] found chlorophyll a to degrade 2.5 times faster than chlorophyll b at 37°C.
Another result from Vongsawasdi *et al.* (2010), who studied the kinetics of chlorophyll degradation and color change of pandanus juice during pasteurization [32], the result showed that chlorophyll degradation followed first order reaction with the rate constant of 0.0282–0.0429 min^{-1} for the pasteurization temperature from 63 to 90°C.

Color changes during thermal processing

In this study, the green color loss (indicated by relative Hunter parameters) during processing time at working temperature was modeled. Since the major color of green, Hunter –a value (redness/greenness) and the combination of parameters a and b determined in terms of –a/b were considered as the visual parameter to describe the green color degradation during thermal processing [25, 24, 21], the kinetic of color changes during thermal processing was carried with the –a value and –a/b ratio. Many authors [13, 12, 32] reported that visual green color loss during heat treatment followed a first–order reaction by using –a value and –a/b ratio as physical properties. The plots of ln($-a/-a_o$) and ln ($-a/b/-a_o/b_o$) varies heating time were displayed in **Figure 3** and **Figure 4**.

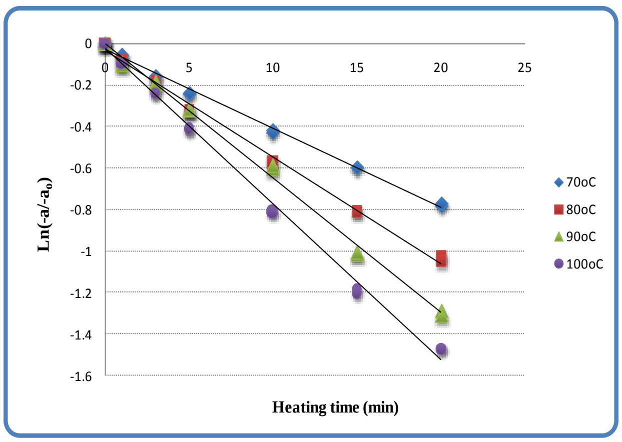

Figure 3: Changes in –a value of bitter gourd during heating at various temperatures

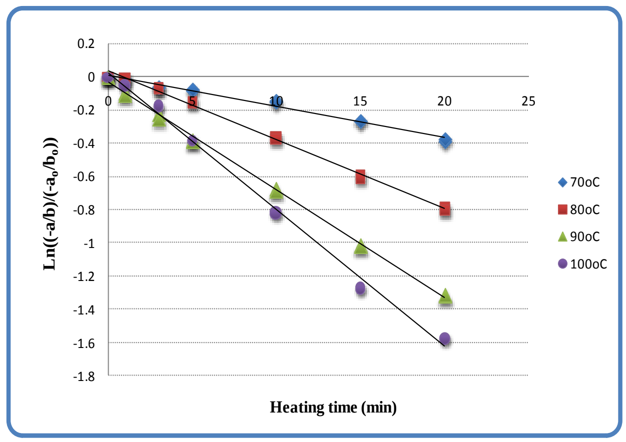

Figure 4: Changes in –a/b ratio of bitter gourd during heating at various temperatures

Figure 3, 4 and **Table 2** showed that the rate constants of ($-a/-a_o$) and ($-a/b/-a_o/b_o$) significantly increased as the temperature increased ($p < 0.05$), indicated that the green color was retained at lower heating temperature. The rate constants for the –a value change ranged from 0.0401 ± 0.0011min^{-1} to 0.0766 ± 0.0017min^{-1}, while the rate constants for –a/b ratio change were from 0.0182 ± 0.0009min^{-1} to 0.0806 ± 0.0026min^{-1}. In the study of Vongsawasdi *et al.* (2010) mentioned above, the kinetic of the changes in color was carried out for L, a, b and –a/b values, all of these parameters followed first order kinetics but there are –a and –a/b values were temperature dependent [32].

Table 2. Kinetic rate constants for color parameter changes during heat treatment of bitter gourd at different temperatures

Parameters	Heating temperature (^oC)			
	70	80	90	100
Rate constant in –a value change $k_{(-a)}$ (min^{-1})	$0.0401^a \pm 0.0011$	$0.0537^b \pm 0.0021$	$0.0649^c \pm 0.0028$	$0.0766^d \pm 0.0017$
Determination coefficients (R^2)	0.990	0.991	0.996	0.995
Rate constant in –a/b ratio change $k_{(-a/b)}$ (min^{-1})	$0.0182^a \pm 0.0009$	$0.0391^b \pm 0.0013$	$0.0674^c \pm 0.0035$	$0.0806^d \pm 0.0026$
Determination coefficients (R^2)	0.985	0.990	0.995	0.995

(Mean ± SD, The values showing different superscripts within a row are significantly different at $p < 0.05$)

Temperature dependency of rate constants

Heating temperature significantly effects on chlorophyll degradation and color change during heat treatment of bitter gourd. The temperature dependence of chlorophyll degradation and visual color loss was also described by the Arrhenius equation as equation (5). The activation energies were calculated by the method of least square approximation and were showed in Table 3.

The results from **Table 3** showed that the temperature–dependence of chlorophylls degradation and visual color loss was described closely by the Arrhenius equation (all $R^2 \geq 0.99$). The activation energy for chlorophyll b degradation (49.96 ± 3.86 kJ/mol) was 1.5 times higher than that for chlorophyll a degradation (32.90 ± 2.68 kJ/mol). Between two temperatures, 0–51^oC, activation energies of 12.5 and 13.0 kcal/mol were reported by Mackinney and Joslyn (1941) [33] for chlorophyll a and b, respectively.

Table 3. Activation energies of chlorophyll degradation and visual color loss of bitter gourd during heat treatment

Parameter	Activation energy (kJ/mol)	R^2
Chlorophyll a	32.90 ± 2.68	0.990
Chlorophyll b	49.96 ± 3.86	0.991
–a value	22.75 ± 1.96	0.996
–a/b ratio	53.55 ± 4.08	0.995

(Mean ± SD)

Activation energies determined on the basis of CIE L, a and b parameters (–a, –a/b) for bitter gourd were 22.75 ± 1.96 and 53.55 ± 4.08 kJ/mol, respectively with the temperature in range of 70–100^oC. Erge *et al.* (2008) published the activation energies for green color loss in green peas with the same temperature range (used –a and –a/b value for determining visual color changes) were 49.75 and 56.04 kJ/mol, respectively [12]. Similarly, Vongsawasdi *et al.* (2010) found the activation energies for a/a_o and $(-a/b)/(-a_o/b_o)$ were 41.49 and 66.14 kJ/mol, respectively [32].

Relationship between chlorophyll content and visual color of bitter gourd

During heat treatment of bitter gourd, the color evidently changed from bright green to dull olive green or olive yellow. This change coincided with chlorophyll degradation of the material. So, maybe, it exist a relationship between the color parameters and chlorophylls content. Regression analysis were carried for –a value and –a/b ratio with total chlorophyll a and b and the plots were displayed in **Figure 5** and **Figure 6**. The dependence of –a value on total chlorophyll a and b followed the exponential equation ($R^2 = 0.97$, $p < 0.01$), while the dependence of –a/b ratio on total chlorophyll a and b obeyed the linear regression ($R^2 = 0.96$, $p < 0.01$).

The green color of vegetables is composed of chlorophyll a responsible for blue–green color and chlorophyll b, giving yellow–green. However, chlorophyll a degrades faster than chlorophyll b as a function of temperature. Therefore, the ratio of chlorophyll a to chlorophyll b decreases with increasing temperature and the green color of vegetables approaches to yellow, gradually. The physical parameters –a and –a/b also decrease as a result of the loss of green color intensity [12]. Seroczyńska *et al*. (2006) [34] found the close relationship between the color parameters and carotenoids content.

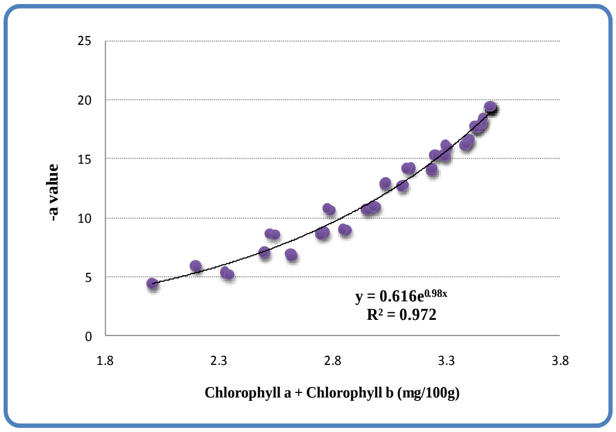

Figure 5: Relationship between –a value and total chlorophyll a and b

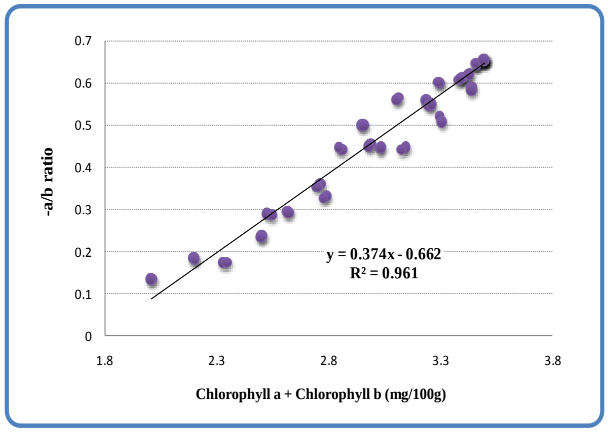

Figure 6: Relationship between –a/b ratio and total chlorophyll a and b

CONCLUSION

The kinetic models for the thermal degradation of chlorophylls in bitter gourd and the stability of green color were validated as being of the first order. The rate constants of all parameters increased with increasing temperature and followed the Arrhenius model. There was significant relationship ($R^2 = 0.96 – 0.97$, $p < 0.01$) between the change of visual color parameters and degradation of total chlorophyll a and chlorophyll b. chlorophylls content and color parameters. Based on the information obtained, the –a value and –a/b ratio from the L, a, and b system of color measurement when subjected to heat treatment proved to be good indicators of the color change during heating of bitter gourd.

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