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Modelling the Kinetics of Peroxidase Inactivation and Colour Changes of Seedless Guava *(Psidium guajava* L.) During Thermal Treatments

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Abstract: The kinetics of thermal inactivation of peroxidase and colour changes in seedless guava (*Psidium guajava* L.) due to hot water blanching were studied in the temperature range of 80-95°C. Peroxidase inactivation kinetics followed a first-order Arrhenius model, where the activation energy and rate of the reaction at a reference temperature of 87.5°C were 101.27±3 kJ mol^{-1} and $0.023\pm4\times10^{-3}s^{-1}$, respectively. Colour was quantified using the L, a, b in Hunter system. The results indicated that colour system parameters (L, a, b) followed a first-order Arrhenius kinetics model with activation energies (E_a) of 120.43±3, 86.45±5 and 100.03±2, $kJmol^{-1}$, respectively. The zero-order kinetic model was applied to total colour difference (TCD) resulting in activation energies of 111.65±5 $kJmol^{-1}$. Good agreement was found between estimated and experimental data in all cases (R^2>0.91).

Key words: Blanching · Colour · Kinetic modeling · Peroxidase inactivation · Seedless guava

Nomenclature:

a	CIE colour space co-ordinate: degree of greenness/redness
A	numerical value of quality factor at time t (peroxidase activity, CIE colour or texture parameters)
b	CIE colour space co-ordinate: degree of blueness/yellowness
C	Residual of peroxidase, L, a, b or TCD parameter at time t
E_a	activation energy (KJ mol^{-1})
K	rate of reaction (s^{-1})
L	CIE colour space co-ordinate: degree of lightness
R	universal gas constant (8.314 J mol^{-1} K^{-1})
t	time (min)
T	absolute temperature (K) *Subscripts*
0	initial (referring to raw product) value
n	Normalised value
ref	at reference temperature

Abbreviation:

TCD	Total colour difference parameter
87.5°C	At the reference temperature of 87.5 °C

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INTRODUCTION

Guava, *Psidium guajava* L., is native to the Caribbean and common throughout all warm areas of tropical America and in the West Indies. The fruits are oblong to pear shape, its skin is thin and the flesh is white, red or salmon-colored and has many seeds in the centre though seedless varieties are also available. In Malaysia commercial guava production began in the mid 1980's and it has been consumed as a fruit for many decades. It has great amount of vitamin C (124 mg/100 g), vitamins A and B. Guavas are useful sources of nicotinic acid, phosphorous and soluble fiber. While having low fat and calories, guavas are cholesterol and sodium free. In order to preserve and commercialize this product, the heat treatment of blanching before further processing such as canning, freezing and dehydration is a necessary step in order to inactivate enzymes responsible for quality changes that occur during distribution and storage. Blanching has some additional advantages like destroying microorganisms and elimination of off-flavour. However, the degree of thermal treatment during blanching process can have adverse effect on sensorial (excessive loss of texture and unwanted changes of colour) and nutritional quality attributes. Many researchers studied these alterations in different fruits. They have observed the dramatic effect of blanching on the degradation of fruit and vegetables nutrient content (namely vitamin C and protein) and antioxidant properties [1-3].

Peroxidases are the most heat stable enzymes in fruits and vegetables and their inactivation is used to indicate the adequacy of blanching. The presence of residual peroxidase in processed products may cause quality changes, such as texture, colour, flavor and nutritional losses, however its role on quality losses during storage period of fruits and vegetables is not clear yet. For these reasons, it is desirable to keep blanching treatment conditions at a level strictly sufficient to cause inactivation of the deleterious enzymes and minimize quality losses.

Colour is a primary consumer perceived characteristic of a product and plays an important role on food acceptance. Furthermore, colour of a processed product is often expected to be as similar as possible to the raw one [4]. Therefore, maintaining the natural colour in processed fruits and vegetables has been a major challenge in food processing. Changes in fruits and vegetables colour can be associated with its previous heat treatment history and is also an indicator of heat treatment severity. Various reactions such as pigment destruction (carotenoids and chlorophylls) and non-enzymatic browning (Maillard) reactions can occur during heating of fruits and vegetables and therefore affect its colour [5-9]. The retention of total colour can be used as a quality indicator to evaluate the extent of deterioration due to thermal processing [10].

Knowledge on degradation kinetics of enzyme inactivation and quality changes including the reaction order, the reaction constant and the energy of activation is essential to predict quality losses during thermal processes. Several researches have been published on modeling of enzyme inactivation and thermal degradation kinetics of colour in different range of temperature. The majority of the published works on enzyme inactivation and colour changes are well described by zero (Eq. (3)), first-order models (Eq. (4)) [11-20] or the fractional conversion (also known as reversible first order model), [21-23]. There is currently no published data for the thermal inactivation of peroxidase and colour changes in seedless guava. Therefore, the aim of this study was to develop mathematical model for peroxidase inactivation and colour changes kinetics of seedless guava during hot water blanching. This information will help to optimize hot water blanching process for seedless guava.

MATERIALS AND METHODS

Materials: Raw seedless guavas *(Psidium guajava* L.) at commercial maturity were purchased from a local market in Serdang, Malaysia. The fruits were washed and peeled. Then, they were cut into cubes (2×2×2 cm). Table 1 presents the initial characteristics of the raw seedless guava. All chemicals used in this study were analytical grade.

Hot Water Blanching Process: Seedless guavas (*Psidium guajava* L.) were blanched in a circulating water bath (Memmert, WNE14. Memmert GmbH + Co. KG,

Table 1: Initial characteristics of raw seedless guava

Colour	
L	82.112±1.232
a	-5.071±0.896
b	21.131±1.543
Water content (%)	90.351±0.681
Water activity (a_w)	0.911±0.004
pH	4.101±0.004
Soluble solids content(°Brix)	8.21±0.900

Germany) maintained at desired temperatures (±0.5°C). Heat inactivation was studied at temperatures ranging from 80 to 95°C, with different times of exposure. After preset times, the samples were removed from the water bath and placed immediately in cooled water (2-5°C) in order to stop thermal inactivation instantaneously. The temperature of the water bath and cooled water was verified with a digital thermometer (Ellab CTD-85, Ellab, Denmark) and a thermocouple (1.2 mm needle diameter constantan-type T). Each experiment was run in triplicates. An unblanched sample was taken as a control.

Enzyme Extraction Procedure: In order to determine the presence of peroxidase in seedless guava and ratio between sample weight (g) and the buffer solution volume (mL), preliminary experiments were carried out. Blanched samples were mixed with cold potassium phosphate buffer in the proportion of 3:25 w/v. Each sample was homogenized in an Ultra-Turrax T25 Janke & Kunkel for 1 min at 13,500 rpm under chilled condition. The homogenate was filtered using filter paper (Whatman No.1). The filtrate was centrifuged in a Beckman Coulter, Avanti J-25 centrifuge with a rotor no.JA14 at 6000×g and 4°C for 20 min with polypropylene tubes. The supernatants were kept on ice until chemical analysis [23].

Determination of Peroxidase Activity: Peroxidase activity was measured according to the method reported by [24]. Peroxidase substrate solution was prepared daily by mixing 0.1 mL guaiacol, 0.1 mL hydrogen peroxide (30%) and 99.8 mL potassium phosphate buffer (0.1mol/L, pH 6.5). Peroxidase assays were conducted by pipetting 0.12 mL of enzyme extract and 3.48 mL of substrate solution in the 10 mm path-length quartz cuvette. Peroxidase activities were measured from the increase in absorbance at 470 nm using an UV/vis spectrophotometer (UV-mini 1240, Shimadzu, Japan). The reaction was monitored for 20 min at 5sec intervals at 25°C. Enzyme activity was calculated from the slope of the initial linear portion of a plot of absorbance vs. time. All experiments were run in triplicates. Residual enzyme activity (REA) in heat-treated samples is expressed as a fraction of initial activity (P_0):

$$\text{Residual enzyme activity (REA)} = P/P_0 \times 100$$

Where; P and P_0 are ΔAbs./min after heat treatment for time t and native enzyme, respectively.

Colour Measurement: Colour of fresh and heat-treated seedless guava cubes were measured using a Minolta tristimulus colorimeter (Minolta, CR-300, Tokyo, Japan) in terms of L-value (lightness), a-value (redness and greenness) and b-value (yellowness and blueness) as an average of three measurements at three different locations. The equipment was calibrated against a standard white reference tile (L = 97.67; a = 0.08 and b = 1.54). From these values, total colour difference (TCD) was calculated according to the following equations:

$$TCD=\sqrt{(L-L_0)+(a-a_0)+(b-b_0)} \quad (1)$$

Where; L_0, a_0 and b_0 are the readings at time zero and L, a and b the individual readings at each processing time.

To minimize the variability between different raw samples, the individual L, a and b values were normalised, dividing the parameters by the corresponding initial values.

$$L(n)=\frac{L}{L_0}; \quad a(n)=\frac{a}{a_0}; \quad b\frac{b}{b_0}; \quad (2)$$

Kinetic Modeling: The zero- (Eq. (3)) and first-order (Eq. (4)) equations were used to describe the enzyme inactivation and colour changes in seedless guava:

$$C=C_0 \pm kt \quad (3)$$

$$C=C_0 \times \exp(-kt) \quad (4)$$

Where; C is the measured value for residual peroxidase activity and colour parameters, C_0 the initial C, t the heating time and k is the reaction rate constant.

The temperature dependence of the rate constant is normally described by an Arrhenius Law:

$$K = K_{ref} \times \exp\left[\frac{-Ea}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right] \quad (5)$$

Where; E_a is the activation energy and K_{ref} is the rate constant at a reference temperature (T_{ref}). T_{ref} was taken as the medium temperature of the range in which this model was used to represent peroxidase inactivation and colour changes (T_{ref} = 87.5°C). The temperature effect can be directly included by substitution of Eq. (5) into kinetic models [23].

Statistical Analysis: Rate constants of seedless guava peroxidase inactivation and colour changes were estimated by non-linear regression analysis, fitting the models of Eq. (3) and Eq. (4) to experimental data. The temperature effect on rate constants was described by the Arrhenius law (Eq. (5)). The pre-exponential factor and the activation energy were estimated directly from experimental data in one-step (quality factor *versus* time, at all temperatures), by performing a global non-linear regression analysis, merging the Arrhenius equation and the kinetic models considered [25]. The reference temperature used was the average value of the range considered (i.e. T_{ref} = 87.5°C), aiming to improve parameter estimation. Parameters' precision was evaluated by confidence intervals at 95% and the quality of the regression was assessed by the coefficient of determination (R^2) and randomness and normality of residuals [26], thus allowing best model selection. Statistica Version 6.0 Software was used for all regression analysis procedures (using least squares estimation and Levenverg–Marquart method, for minimising the sum of squares of the deviations between experimental values and the ones predicted by the mathematical model). An analysis of variance was performed to determine significance of differences among treatments for peroxidase activity and colour changes.

RESULTS AND DISCUSSION

Peroxidase Inactivation: In the hot water blanching study, it was observed that the time required for the inactivation of peroxidase changed with the temperature gradients applied. The enzyme inactivation was significantly affected ($P < 0.05$) by the time and temperature of the blanching process (Table 2). The residual peroxidase activities in seedless guava against blanching time for different processing temperatures are presented in Fig. 1. Inactivation kinetic models were tested for its applicability to the thermal inactivation data for seedless guava. Among them, the monophasic first-order kinetic model yield good R^2 values (above 0.96) in the range of temperatures tested. Based on the examination of the residuals, this proved to be the most adequate model, once the distribution of residuals has no visual tendency (were randomly distributed around zero). Fig. 2 shows the residuals plot (with no tendency) for the experimental data.

Table 2: Analysis of variance for peroxidase inactivation in seedless guava by thermal treatment

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	1	1.71776	2.22879	2.22879	60.32	0.000
Temperature	3	0.52172	0.52172	0.17391	4.71	0.012
Error	20	0.73899	0.73899	0.03695		

Table 3: Kinetic parameters and corresponding confidence intervals at 95% of seedless guava peroxidase inactivation and colour changes due to hot water blanching

		Kinetic parameter			
Quality factor		C_0	$K_{87.5°C}(s^{-1})$	$E_a(KJmol^{-1})$	R^2
Peroxidase inactivation		1.00±0.02	$0.023±4\times10^{-3}$	101.27±3.00	0.99
Colour changes	L	0.99±0.01	$0.002±4\times10^{-4}$	120.43±3.00	0.96
	a	0.98±0.03	$0.004±1\times10^{-3}$	86.45±5.00	0.91
	b	0.99±0.02	$0.004±8\times10^{-4}$	100.03±2.00	0.97
	TCD	0	$0.150±5\times10^{-3}$	111.65±5.00	0.97

Consequently, the monophasic first-order kinetic model was selected to model the kinetics of peroxidase inactivation in seedless guava. The model parameters (activation energy, the rate of the reaction at the reference temperature of 87.5°C) was estimated as, E_a = 101.27±3 kJ mol^{-1} and $K_{87.5°C} = 0.023±4\times10^{-3}$ s^{-1}, respectively (Table 3).

Monophasic behavior of the enzyme inactivation at high temperatures could be due to the rapid inactivation of the heat-labile fraction of the enzyme during the first seconds of treatment, so the observed kinetics would correspond to the inactivation of the heat-resistant fraction of peroxidase. Similarly, the peroxidase inactivation in different vegetables, such as carrots, potatoes, tomato, green beans, green asparagus and pumpkin has been reported to follow a first-order model to describe the enzyme inactivation [11, 12, 27-30].

Colour Changes: The L, a and b parameters were significantly affected ($P < 0.05$) by the time and temperature of the blanching process. Enzymatic browning is a serious problem because the oxidative enzymes, such as peroxidase and polyphenolase, may cause browning accompanied by changes in colour, flavor and nutritive value [5]. During thermal treatment, those enzymes were inactivated, but many other reactions can take place affecting colour. Chlorophyll and carotenoid pigments decomposition [31-33] and formation of brown pigments by non-enzymatic browning (Millard) reactions are the most common [14, 34].

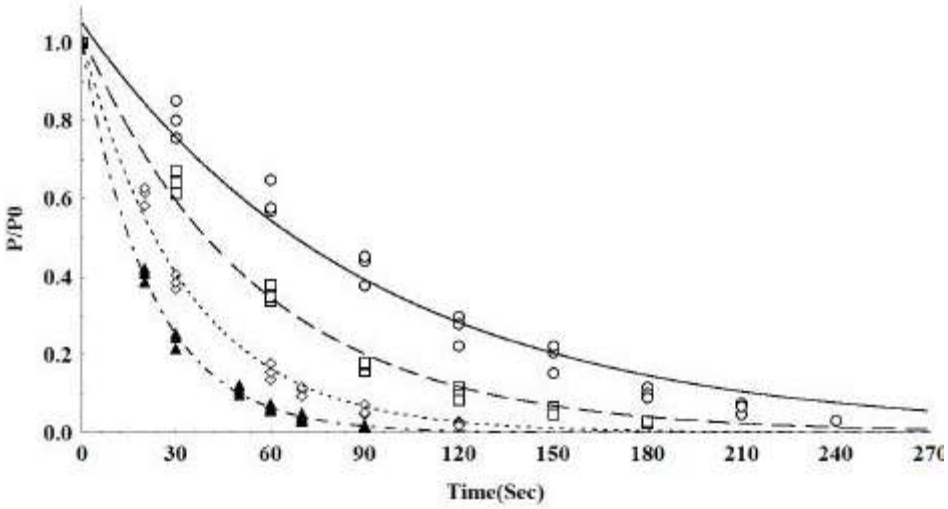

Fig. 1: Seedless guava peroxidase inactivation during blanching process (□experimental data at 80°C; □ at 85°C; ◊ at 90°C; ▲ at 95°C). The lines represent model fits to experimental data

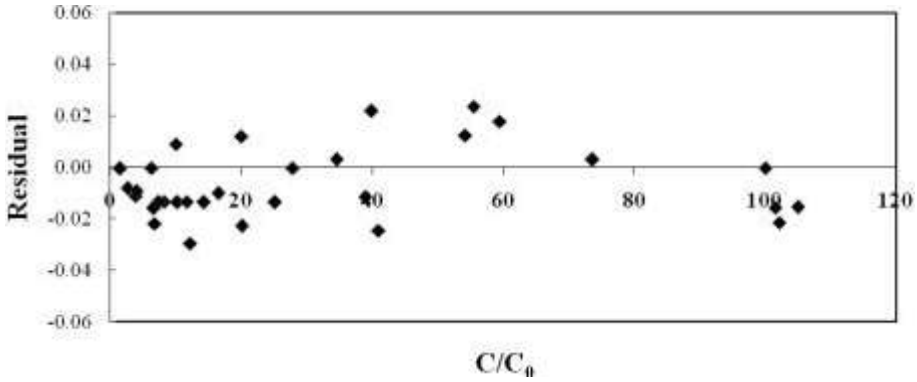

Fig. 2: Plot of residual for C/C_0 experimental data against the predicted values of the model

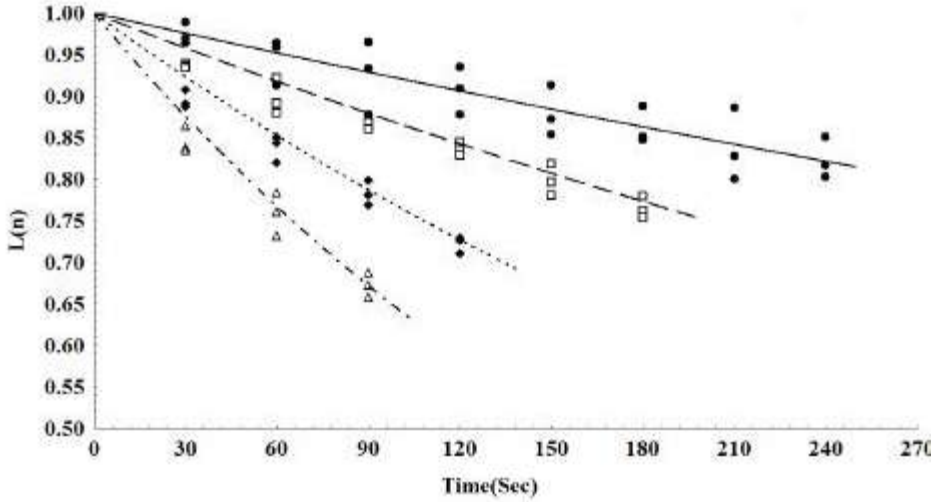

Fig. 3: Seedless guava L(n) value changes during blanching process (● experimental data at 80°C; □ at 85°C; ♦ at 90°C; △ at 95°C). The lines represent model fits to experimental data

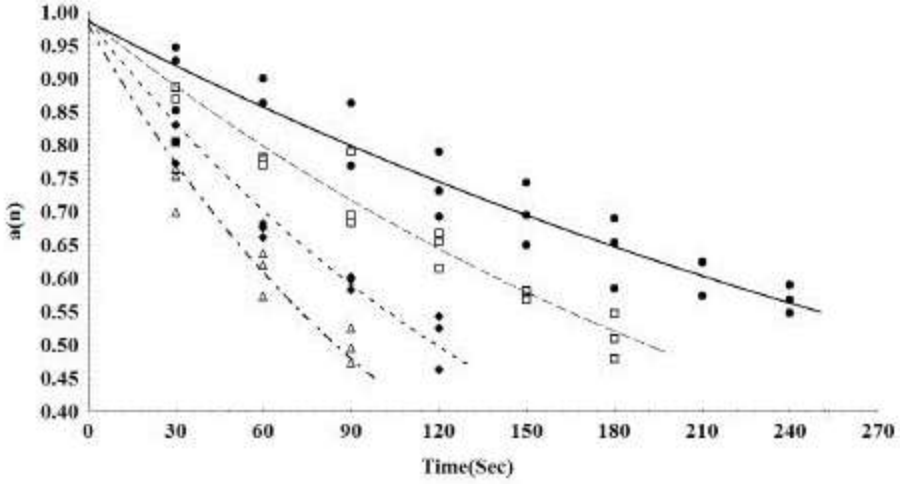

Fig. 4: Seedless guava a(n) value changes during blanching process (● experimental data at 80°C; □ at 85°C; ♦ at 90°C; △ at 95°C). The lines represent mo del fits to experimental data

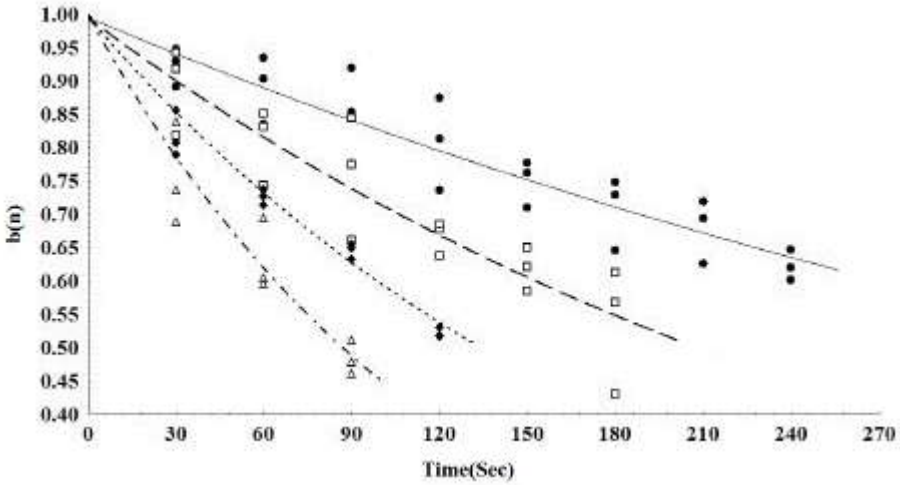

Fig. 5: Seedless guava b(n) value changes during blanching process (● experimental data at 80°C; □ at 85°C; ♦ at 90°C; △ at 95°C). The lines represent model fits to experimental data

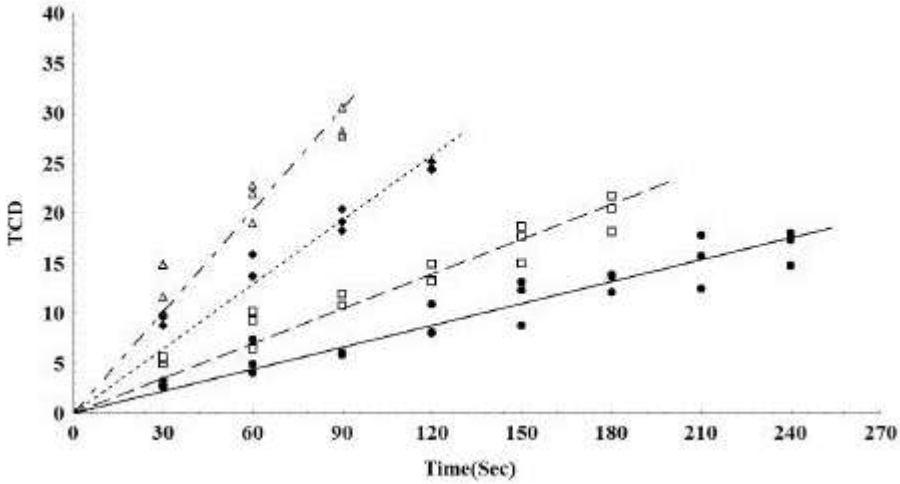

Fig. 6: Seedless guava TCD value changes during blanching process (■ experimental dasta at 80°C; □ at 85°C; ♦ at 90°C; △ AT 95°C). The lines represent model fits to experimental data

The normalized lightness L(n) values decreased with treatment time can be observed in Fig. 3. Since L is a measure of the colour in the light-dark axis, this falling value indicates that the samples were turning darker. In the present study, variation of the normalised L(n) with the treatment time at different temperatures was well fitted (R^2=0.96) to a first order kinetic model with Arrhenius temperature dependence (Eq. (4)).

Barreiro *et al.* [17] and Avila *et al.* [21] applied the same kinetic model for apple, peach, prune purees and tomato paste treated thermally.

Figs. 4 and 5 show changes in the normalized a(n) and b(n) parameters with the time and temperature of treatments. The colorimetric a(n) and b(n) parameter tended to decrease in value with time and treatment temperature. A first order kinetic model with Arrhenius temperature dependence (Eq. (4)) fitted well the experimental data for the a(n) and b(n) parameters (R^2=0.91-0.97), respectively [15, 17, 18].

One of the best parameters for describing the colour variation is the total colour difference (TCD) since it is a combination of parameters L, a and b. Fig. 6 shows the variation of this parameter with the treatment time for the different temperatures. An Arrhenius zero order kinetic model (Eq.(3)) was used to model the thermal degradation of TCD parameter(R^2=0.97). Aguerre and Suarez [13]; Rhim *et al.* [14] and Barreiro *et al.* [17] applied the same kinetic model for corn, grape juice, double concentrated tomato paste treated thermally.

The high activation energies of all colour parameters (values between 86.45 and 120.43 kJ mol^{-1}) are related to seedless guava temperature sensitivity. In all cases normality and randomness of residuals were verified and coefficient of determination, R^2, was satisfactorily high. Tables 1 present the estimated kinetic model parameters at corresponding 95% confidence intervals, respectively for hot water blanching treatment.

CONCLUSION

The effect of blanching conditions on the activity of the peroxidase enzyme in seedless guava (*Psidium guajava* L.) was studied. At the range of temperatures (80 – 95°C), the heat-labile fraction was rapidly inactivated and the kinetic behavior would correspond to the heat-resistant fraction that followed monophasic first-order inactivation kinetics. The colour changes were well described by zero- and first-order kinetic model. The Arrhenius model described the temperature dependence of the reaction rate constant of all the factors considered. With these models and the estimated kinetic parameters, it would be possible to predict the residual peroxidase activity and colour changes for a given set of time–temperature conditions. This work will help to design the seedless guava (*Psidium guajava* L.) blanching conditions.

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