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Research Article

Kinetics of chlorophyll degradation in pandanus juice during pasteurization

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Abstract

The objectives of this research are to study the kinetics of chlorophyll degradation and colour change of pandanus juice during pasteurization. Pandanus juice was prepared using pandanus leaves and water at the ratio of 1:4 (w/w). Total soluble solid of the juice extract was adjusted to 12±2°Brix before the juice underwent pasteurization. In this study, 4 pasteurization temperatures (63, 70, 80 and 90°C) were investigated at the constant pasteurization time of 30 minutes. The results showed that pasteurization conditions had significant effects on juice appearance ($p \leq 0.05$). Chlorophyll content of pandanus juice decreased as pasteurization temperature and time increased. The colour changes in terms of L, a and b were also determined. Kinetics study revealed that chlorophyll degradation followed first order reaction with the rate constant and activation energy of 0.0282-0.0429 min^{-1}, 14.83 $kJmol^{-1}$, respectively. The changes in L/L_0, a/a_0, b/b_0 and $(-a/b)/(-a_0/b_0)$ also followed first order kinetics with the rate constant of 0.0079-0.0112, 0.0052-0.0196, 0.0035-0.0047 and 0.0017-0.0149 min^{-1}, respectively and their activation energies were 11.35, 41.49, 10.69 and 66.14 $kJmol^{-1}$, respectively.

Keywords: beverages, *Pandanus amaryllifolius*, functional food, colour change, Thailand.

Introduction

The recent trend in the food industry towards safe and highly nutritious food is driven by consumers requiring food with pharmaceutical functions. Pandanus juice is one of the

popular beverages in Thailand because it is rich in Ca, Mg and P. It has a particularly high vitamin A and chlorophyll content as well [1]. One of the most important medicinal properties of pandanus juice is relief of anemia [2]. The juice, prepared in water is said to diminish inflammation and is a cure for coughing. It is also used to cure respiratory disorders such as asthma, protect against some toxins and ameliorate some drug side effects [2]. For these reasons, pandanus juice is attractive to consumers. However, fresh juice has a quite limited shelf life due to microbial spoilage. Pasteurization is one approach to overcome this problem. The selection of pasteurization conditions is determined by factors such as economics and effects on product quality. According to Schwartz and Elbe [3], chlorophyll is highly susceptible to degradation during processing which results in colour changes in the product. Major chemical degradation routes are associated with pheophytinization, epimerization, pyrolysis and hydroxylation [4]. Since product colour is of critical importance to consumer acceptability, it is necessary to prevent this chloropigment degradation during heat treatment. Much of the research has reported on chloropigment degradation of various vegetables [3, 5, 6] and numerous studies have demonstrated that the chlorophyll degradation follows a first-order reaction kinetics model [3, 5, 7]. However, there are no reports on the degradation of chrophyll in pandanus juice. Therefore the purpose of this research is to understand degradation kinetics during the heating period as well as its effects on colour of pandanus juice. The information obtained from this research will strengthen the potential use of this juice as a natural functional beverage.

Materials and Methods

Preparation of pandanus juice

Pandanus leaves were purchased from a local market in Bangkok, Thailand. The leaves were hand-washed in tap water and blanched in boiling water for 4 min. After chopping into small pieces, they were homogenized with water (1:4 w/w) (Sharp, Japan) and filtered through 4-layers of cheesecloth. Total soluble solid of extract was adjusted to 12 ± 2 ^{o}Brix. The juice was then kept at 4 ± 1^{o}C until used.

Heat treatment

Chlorophyll degradation and colour changes of pandanus juice were studied in triplicate, in a series of thin wall glass tubes (length 30 cm, inner diameter 8 mm, wall thickness 1 mm) at constant temperatures of 63, 70, 80 and 90^{o}C (Jurabo, Germany). The rise in temperature time for each condition was less than 1 min and the heating time was measured from the time the product temperature reached processing temperature. Samples were removed every 5 min for a period of 30 min and immediately cooled in order to stop the reaction. Chlorophyll content and colour changes of pandanus juice were then determined.

Determination of chlorophyll content

Total chlorophyll content was determined by modification of the Vernon method [8]. Chlorophyll in 5 ml of pandanus juice was extracted with 20 ml of 80% (v/v) acetone under subdued light for 15 min. The homogenate was filtered through Whatman No. 42 paper and centrifuged at 2500 rpm for 10 min (Hettich GMBH, Germany). The absorbance of supernatant was read at 665 and 649 nm. (Labomed, USA). The amount of chlorophyll was calculated from:

$$\text{Total chlorophyll content (mg/l)} = 6.45(A_{665}) + 17.72(A_{649})$$

where A_{665} and A_{649} are the absorbance of samples at 665 and 649 nm, respectively.

Colour measurement

Colour changes of pandanus juice were determined by measuring the transmittance using a colour spectrophotometer (Hunterlab, Ultrascan XE/X7,USA) and reported in terms of L (lightness), a (redness or greenness) and b (yellowness or blueness). The instrument was calibrated against white tile (L= 99.12, a= -0.26 , and b=-0.34) before the measurement.

Kinetics determination

The rate constants of chlorophyll degradation and colour changes were estimated by regression of the experimental data. Activation energy and pre-exponential factors were determined from the Arrhenius equation.

$$k = k_0 e^{\frac{-Ea}{RT}}$$

Where Ea is the activation energy ($kJmol^{-1}$), k is the rate constant, k_0 is the pre-exponential factor, R is the universal gas constant ($8.314x10^{-3}$ $kJK^{-1}mol^{-1}$) and T is the absolute temperature (K).

Data analysis

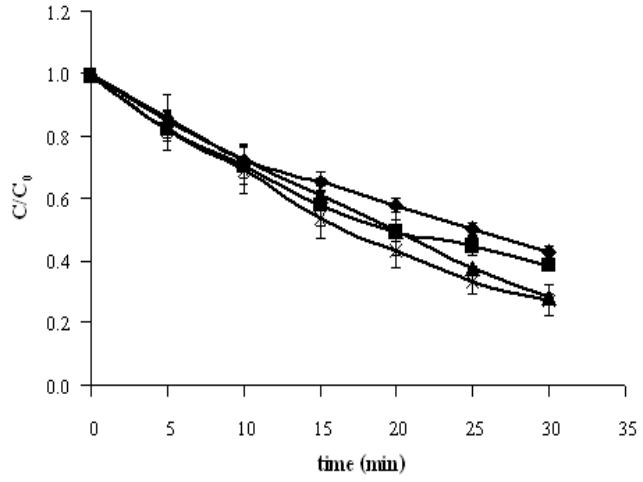
Analysis of variance (ANOVA) was used to determine the effect of treatments on the dependent variables. Means were compared using Duncan's Multiple Range Test at $p \leq 0.05$.

Results and Discussion

The effect of pasteurization temperature (63-90^{o}C) on the degradation of chlorophyll and colour changes in pandanus juice was investigated. Figure 1 illustrates the ratio of chlorophyll content to its original content. The results showed that chlorophyll content decreased with temperature at any given time ($p \leq 0.05$). Higher temperature resulted in lower pigment concentration. As shown in Figure 1, relative chlorophyll content of 0.28 ± 0.04 was observed after heating the juice at 90^{o}C for 30 min whereas that of 0.43 ± 0.02 was found after heating the juice at 63^{o}C for the same period of time. During thermal processing, chlorophyll undergoes isomerization. The central magnesium atom in the porphyrin ring is substituted by two hydrogen atoms, thus leading to pheophytin and pheophobide formation [4, 9, 10, 11]. Hence, chlorophyll content of pandanus juice significantly decreased.

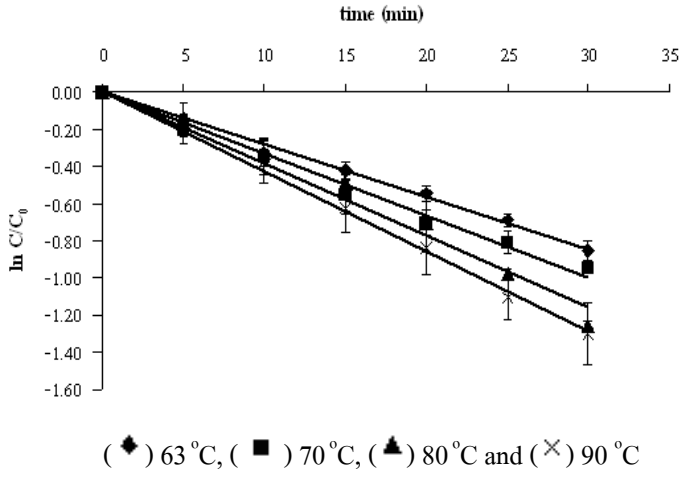
The first order kinetics model has been used to describe the degradation of chlorophyll in vegetables [3, 5, 7]. In this study, it was verified that chlorophyll degradation followed the first order kinetics model (Figure 2). Table 1 shows that the degradation rate of chlorophyll increased from 0.0282 ± 0.0015 min^{-1} at 63^{o}C to 0.0429 ± 0.0057 min^{-1} at 90^{o}C. This means that the green pigment was denatured more quickly at higher temperature. Weemaes *et al* [12], studied the kinetics of chlorophyll degradation of broccoli juice during heat process at temperatures of 80-120^{o}C. They found that this reaction was temperature dependent with the rate constant of 0.0085-0.0943 min^{-1}. The deviation of estimated degradation rate constant in this study might be due to variation in overall composition of plant juice tested. A similar trend was observed by Koca, Karndiniz and Burdurlu [13], for the chlorophyll of green pea where the half-life of pigment decreased when blanching temperature increased.

Arrhenius equation was used to explain the correlation between rate constant and temperature. Activation energy was calculated on the basis of linear regression analysis of natural logarithms of rate constants against reciprocal of absolute temperatures, 1/T in K. From Table 2, activation energy for chlorophyll degradation in pandanus juice was 14.83 kJ mol^{-1}. The estimated activation energy value in this study was lower than the values estimated by Weemaes *et al* [12], who revealed that the activation energy of total chlorophyll in broccoli juice was 69.04 kJ mol^{-1}.



(♦) 63 °C, (■) 70 °C, (▲) 80 °C and (×) 90 °C

Figure 1. Relative chlorophyll content (C/C_0) during thermal process at various temperatures for 30 min.



(♦) 63 °C, (■) 70 °C, (▲) 80 °C and (×) 90 °C

Figure 2. First order thermal degradation of chlorophyll (C/C_0) in pandanus juice during thermal process at various temperatures for 30 min.

In subsequent study, relation between the green colour loss (indicated by relative Hunter parameters) and processing time at working temperature are shown in Figures 3a-3c. To better describe the total colour of pandanus juice, the combination of parameters a and b

were determined in terms of –a/b [14] and its relation with pasteurization conditions was shown in Figure 3d. As shown by the results obtained, L/L_0, a/a_0, b/b_0 and $–a/b/-a_0/b_0$ significantly increased, corresponding to increase in temperature and time. The juice colour evidently changed from bright green to dull olive green or olive yellow. This change coincided with chlorophyll degradation of the juice. Since the L/L_0 of the juice increased with the processed conditions, it is imperative that pheophobide might be degraded to fluorescent compound [11].

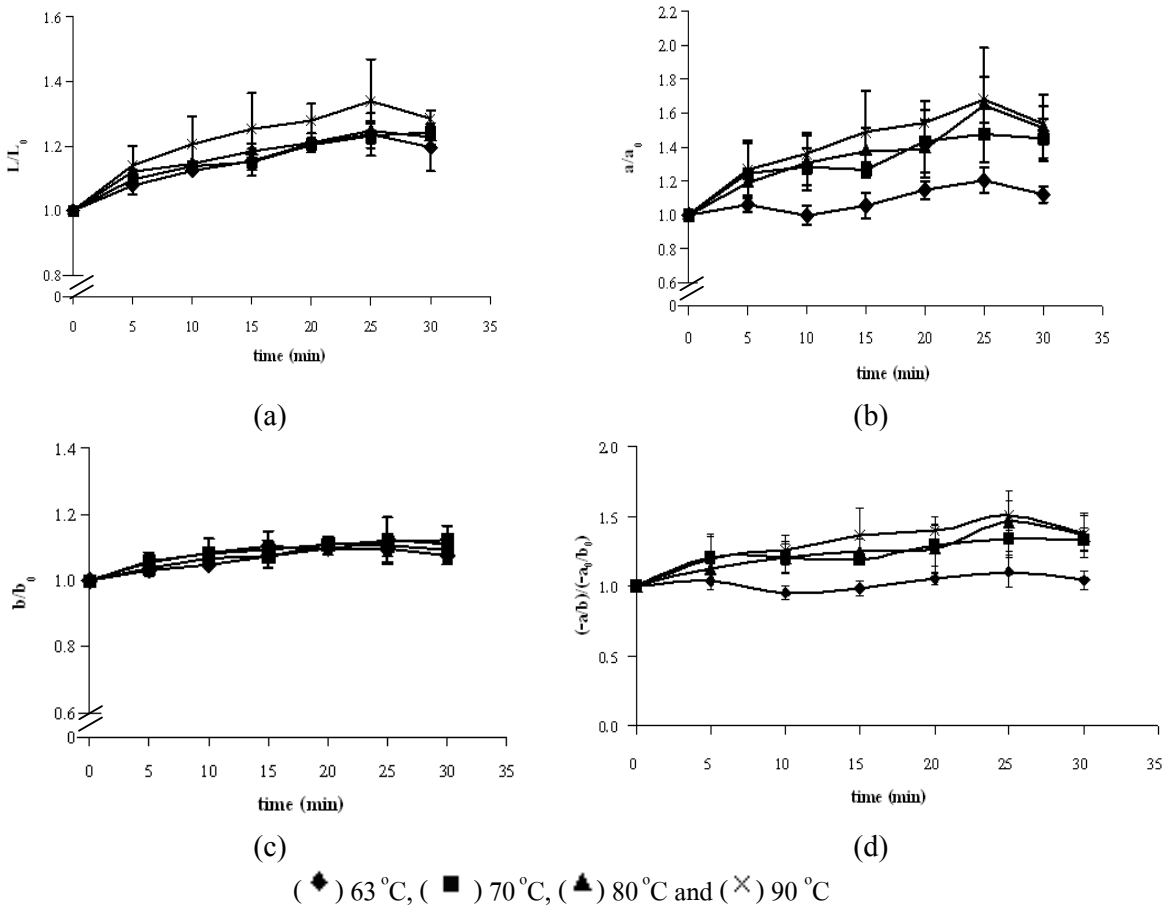


Figure 3. Relative colour changes (L/L_0, a/a_0, b/b_0 and $(-a/b)/(-a_0/b_0)$) of pandanus juice during thermal process at various temperatures for 30 min.

The green colour loss at each heating condition, on the basis of changes in L/L_0, a/a_0, b/b_0 and $(-a/b)/(-a_0/b_0)$ followed the first-order reaction (Figure 4). Rate constants of a/a_0 and $(-a/b)/(-a_0/b_0)$ significantly increased as the temperature increased ($p \leq 0.05$) while those of L/L_0 and b/b_0 were not affected by working temperature ($p > 0.05$) (Table 1). This implied that only a/a_0 and $(-a/b)/(-a_0/b_0)$ were temperature dependent. Increasing rate constant of those two parameters with increasing temperature indicated that the green colour was retained at lower heating temperature. Temperature-dependence of visual colour loss was also described by the Arrhenius equation. The activation energies for a/a_0 and $(-a/b)/(-a_0/b_0)$ were 41.49 and 66.14 $kJmol^{-1}$, respectively (Table 2) while those for L/L_0 and b/b_0 were 11.35 and 10.69 $kJmol^{-1}$, respectively. The high activation energy of a/a_0 and $(-a/b)/(-a_0/b_0)$ suggested that these two parameters were much more susceptible to heat than L/L_0 and b/b_0.

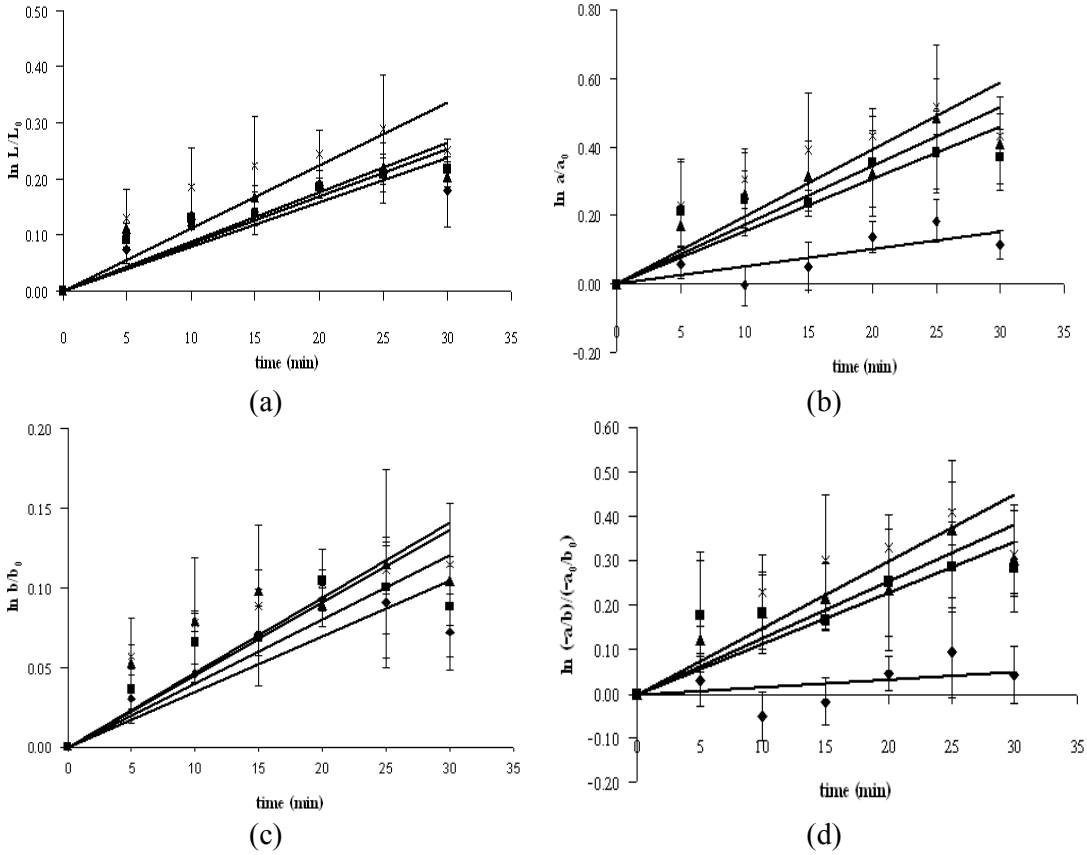


Figure 4. First-order thermal degradation of colour variables (L/L_0, a/a_0, b/b_0 and $(-a/b)/(-a_0/b_0)$) during thermal processing at various temperatures for 30 min.

Table 1. Degradation rate constant (k) of chlorophyll and colour variable (L/L_0, a/a_0, b/b_0 and $(-a/b)/(-a_0/b_0)$) of pandanus juice during thermal processing at temperatures of 63-90°C.

Item	Rate constant (min^{-1})			
	63°C	**70°C**	**80°C**	**90°C**
Chlorophyll	$0.0282^{a}\pm0.0015$	$0.0333^{ab}\pm0.0034$	$0.0385^{bc}\pm0.0004$	$0.0429^{c}\pm0.0057$
L/L_0 ns	0.0079 ± 0.0013	0.0085 ± 0.0006	0.0088 ± 0.0008	0.0112 ± 0.0020
a/a_0	$0.0052^{a}\pm0.0010$	$0.0154^{b}\pm0.0041$	$0.0172^{b}\pm0.0019$	$0.0196^{b}\pm0.0036$
b/b_0 ns	0.0035 ± 0.0007	0.0040 ± 0.0012	0.0046 ± 0.0011	0.0047 ± 0.0009
$(-a/b)/(-a_0/b_0)$	$0.0017^{a}\pm0.0015$	$0.0114^{b}\pm0.0036$	$0.0126^{b}\pm0.0016$	$0.0149^{b}\pm0.0034$

* means with different letters (a,b,...) in the same row are significantly different ($p\leq0.05$)

ns means in the same column are not significantly different ($p>0.05$)

Table 2. Activation energies (Ea) and pre-exponential factors (k_0) of chlorophyll and colour degradation (L/L_0, a/a_0, b/b_0 and $(-a/b)/(-a_0/b_0)$) of pandanus juice.

Item	Ea ($kJmol^{-1}$)	k_0 (min^{-1})
Chlorophyll	14.83	5.91
L/L_0	11.35	0.45
a/a_0	41.49	21234.87
b/b_0	10.69	0.17
$(-a/b)/(-a_0/b_0)$	66.14	6.42×10^7

Conclusion

The kinetic models for the thermal degradation of chlorophyll in pandanus juice and the stability of green colour were validated as being of the first order. The rate constants of both parameters increased with increasing temperature**.** Based on the information obtained, chlorophyll and colour stability of pandanus juice can be predicted and the appropriate processing conditions can be adjusted to suite this.

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