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Effect of pH on chlorophyll degradation and colour loss in blanched green peas

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Abstract

Effect of pH on the chlorophyll degradation and visual green colour loss in blanched green peas were studied at 70, 80, 90 and 100 °C in buffered solutions of pH 5.5, 6.5 and 7.5. The degradation of chlorophylls *a* and *b* followed a first-order reaction and the temperature-dependence of these reactions was modelled by the Arrhenius equation. The activation energies ranged from 4.80 ± 0.91 to 14.0 ± 0.71 kcal mol^{-1} for chlorophyll *a* and from 6.84 ± 0.29 to 11.0 ± 1.06 for chlorophyll *b* with varying pH values. The visual green colour degradation, as represented by the change of the $-a$ (greenness), the ratio $-a/b$ and hue (h) values measured by tristimulus colorimeter, also followed a first-order reaction. Activation energies for $-a$ values ranged from 8.13 ± 0.71 to 12.0 ± 1.07 kcal mol^{-1}, and for $-a/b$ values ranged from 8.77 ± 1.34 to 12.0 ± 1.07 kcal mol^{-1} with varying pH values at 70, 80, 90 and 100 °C.

Keywords: Chlorophyll; Colour; Kinetics; HPLC; Green peas; pH; Blanching

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1. Introduction

Chlorophylls, the pigments responsible for the characteristic green colour of fruits and vegetables, are highly susceptible to degradation during processing, resulting in colour changes in food (Schwartz & von Elbe, 1983). The major chlorophylls in plants include chlorophyll *a* and chlorophyll *b*, which occur in the approximate ratio of 3:1 (von Elbe & Schwartz, 1996). Chlorophyll *a* has a methyl group at the C-3 carbon, while a formyl group is bonded to the same carbon atom in chlorophyll *b*. In addition to structural differences between chlorophyll *a* and *b*, their thermal stabilities are also different. Chlorophyll *a* was reported to be thermally less stable than chlorophyll *b* (Buckle & Edwards, 1970; Canjura, Schwartz, & Nunes, 1991; Lajollo, Tannenbaum, & Labuza, 1971; Schwartz & Lorenzo, 1991; Schwartz & von Elbe, 1983; Tan & Francis, 1962).

Chlorophyll retention has been used as a measure of quality in green vegetables (Sweeney & Martin, 1961). It is well-known that the excessive heating of food products causes considerable losses in the organoleptic quality of food (Hayakawa & Timbers, 1977). Blanching inactivates chlorophyllase and enzymes responsible for senescence and rapid loss of green colour. However, chlorophyll degradation is initiated by damaged tissue during blanching and other processing steps (Heaton & Marangoni, 1996; Tijkens, Barringer, & Biekman, 2001).

Chlorophylls are susceptible to many chemical or enzymatic degradation reactions. The simultaneous actions of enzymes, weak acids, oxygen, light and heat can lead to the formation of a large number of degradation products. Major chemical degradation routes are associated with pheophytinization, epimerization, and pyrolysis, and also with hydroxylation, oxidation or photo-oxidation, if light is implicated (Mangos & Berger, 1997). There is general agreement that the main cause of green vegetable discolouration during processing is the conversion of chlorophylls to pheophytins by the influence of pH. The green colour of vegetables turns to an olive green when heated

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or placed in acidic conditions (Gold & Weckel, 1959; Gunawan & Barringer, 2000). During this reaction, hydrogen ions can transform the chlorophylls to their corresponding pheophytins by substitution of the magnesium ion in the porphyrin ring (Minguez-Mosquera, Garrido-Fernandez, & Gandul-Rojas, 1989). The conversion of chlorophyll to pheophytin and pheophorbide results in a change from bright green to dull olive-green or olive-yellow, which is ultimately perceived by the consumer as a loss of quality (Gupte, El-Bisi, & Francis, 1963).

A number of attempts have been made to preserve chlorophylls during heat processing through the application of pH control (Blair & Ayres, 1943; Gupte & Francis, 1964), high-temperature short time processing (Clydesdale & Francis, 1968; Schwartz & Lorenzo, 1991; Tan & Francis, 1962), or a combination of high-temperature short time processing with pH adjustments (Buckle & Edwards, 1970; Gupte & Francis, 1964). Other improvements in colour have involved the production of the more heat-stable chlorophyllides (Loef & Thung, 1965). Alkalizing agents in blanch and brine solutions, such as sodium bicarbonate, hexametaphosphate, disodium glutamate, sodium hydroxide, and magnesium hydroxide, have been used to raise the pH of green vegetables and therefore, retain chlorophyll after processing (Blair & Ayres, 1943; Gilpin, Sweeney, Chapman, & Eisen, 1959).

Since chlorophyll stability is known to be affected by pH and colour is one of the most important quality attributes of vegetable products, numerous studies have been conducted to investigate the colour changes or degradation of chlorophylls during heating (Chen & Chen, 1993; Schwartz, Woo, & von Elbe, 1981) and it is reported that the chlorophyll degradation follows a first-order reaction kinetic model (Canjura et al., 1991; Gold & Weckel, 1959; Schwartz & von Elbe, 1983; Schwartz et al., 1981; Steet & Tong, 1996).

Earlier research relied on the use of spectrophotometric and colorimetric techniques to determine the kinetic parameters of chlorophyll degradation in model systems of green vegetables (Gold & Weckel, 1959; Gupte & Francis, 1964). In this study, the degradation of chlorophyll *a* and chlorophyll *b* were determined by using HPLC, as reported by the previous studies (Canjura & Schwartz, 1991; Mangos & Berger, 1997; Ryan-Stoneham & Tong, 2000; Schwartz & von Elbe, 1983; Steet & Tong, 1996; Weemaes, Ooms, Van Loey, & Hendrickx, 1999) and also by the CIE-$L^*a^*b^*$ system which is frequently used as a versatile and reliable method to assess the colour of fruit and vegetables. (Barrett, Garcia, Russell, Ramirez, & Shirazi, 2000; Gnanasekharan, Shewfelt, & Chinnan, 1992; Gunawan & Barringer, 2000). The $-a$ value has been used as a physical parameter to represent greenness in colour measurement (Cano & Marin, 1992; Weemaes et al., 1999). Additionally, other researchers (Gold & Weckel, 1959; Gunawan & Barringer, 2000; Hayakawa & Timbers, 1977) have monitored changes in chlorophyll content by the ratio $-a/b$ for canned green peas, canned green beans and blanched and frozen broccoli, respectively.

Since the green colour is one of the major sensory characteristics in determining the final quality of thermally processed green vegetables, it is important to prevent or at least minimise chlorophyll degradation during thermal processing in the food industry. pH control, as mentioned previously, is one of the methods for preventing green colour loss. Because the pH effect on chlorophyll degradation has not been studied extensively, the present investigation was conducted for determining the effect of pH on kinetic parameters in green colour changes of vegetables. In this research, kinetic parameters for chlorophyll *a*, chlorophyll *b* and visual green colour degradation as a function of pH in buffered solutions at pH 5.5, 6.5 and 7.5, in blanched green peas at 70, 80, 90 and 100 °C, have been studied by high performance liquid chromatography and tristimulus colorimetry.

2. Materials and methods

2.1. Materials

2.1.1. Reagents

Chlorophyll *a* and *b* standards were purchased from Sigma–Aldrich Co. (St. Louis, MO., USA). All analyses were performed by using HPLC grade (Merck, Darmstadt, Germany) solvents.

2.1.2. Sample preparation

Fresh green peas were supplied by one of the biggest canning companies in Turkey and were stored at 0 °C under 95% relative humidity in polyethylene bags prior to the analyses.

2.1.3. Blanching treatment

Dehulled green peas in cheesecloth bags were blanched in buffered solutions of pH 5.5, 6.5 and 7.5 at 70, 80 and 90 °C in a water-bath (Memmert, Schwabach, Germany) and at 100 °C in an oil-bath (Normschliff, Wertheim). Buffered solutions were prepared by combining 0.1 M citric acid with 0.1 M disodium hydrogen phosphate (McIlvaine's buffer) to the desired pH ± 0.05. An overview is given of the sampling frequency at different pH values and temperatures in Table 1. At the end of given heating periods,

Table 1
Sampling frequency at different pH–temperature combinations

T (°C)	Time (min)		
	pH 5.5	pH 6.5	pH 7.5
70	0, 10, 20, 30, 40, 50, 60	0, 10, 20, 30, 40, 50, 60, 70	0, 20, 50, 60, 80, 100, 120
80	0, 5, 10, 15, 20, 25, 30	0, 5, 10, 15, 25, 35, 45, 55	0, 10, 20, 30, 40, 60, 70, 80
90	0, 5, 10, 15, 20, 25	0, 5, 10, 15, 20, 30, 40	0, 10, 20, 30, 40, 60
100	0, 3, 6, 9, 12, 15, 20	0, 3, 6, 9, 12, 15, 20	0, 3, 5, 15, 25, 35, 45, 55, 65, 75

samples were immediately cooled under tap water. Blanched peas were allowed to drain and mashed in a mortar before conducting the following methods of analyses. Blanching treatments were carried out with two replicates at selected time–temperature combinations.

2.2. Methods

2.2.1. Colour analysis

The CIE-Lab a^*, b^* and hue (h) values for each sample were measured by a Minolta Chromameter (Model CR-300, Osaka, Japan). For evaluating the colour changes of processed green vegetables, $-a$, h and the ratio of $-a/b$ parameters were taken into account. Four measurements were taken for each sample.

2.2.2. Pigment extraction

The chlorophylls were extracted from the green peas by adding 18.8 ml of acetone to 5 g of pea puree (Canjura et al., 1991). The mixture was homogenized by using an Ultra-Turrax homogenizer (IKA Werke, Labortechnik, Staufen, Germany) for 2 min. The slurry was then filtered under vacuum through Whatman No. 42 filter paper. The filtrate was brought to volume with 80% acetone in a 25-ml volumetric flask. Prior to injection into the HPLC, the sample was filtered through a 0.45 μm durapore membrane filter (HVHP Millipore Co., Watford, Ireland) and 20 μl aliquots of filtrate were immediately analyzed for the determination of chlorophylls.

2.2.3. HPLC analyses

The chlorophyll pigments were separated on a Zorbax ODS column (5 μm, 250 × 4.6 mm i.d.) (Agilent Technologies, USA) preceded by a Zorbax ODS guard column (5 μm, 12.5 × 4.6 mm) according to a modified version of the method described by Schwartz and von Elbe (1983). A waters 510 HPLC pump (Millipore Co., Milford) was used. A waters 486 UV–VIS detector (Millipore Co., Milford, USA) was set at 430 nm for the detection of chlorophyll pigments. Integration and data storage were performed with Millenium 2010 Chromatography software (Millipore Co., Milford, USA). Chlorophyll a and chlorophyll b were eluted with an isocratic mobile phase of ethyl acetate:methanol:water (50:37.5:12.5) at a flow rate of 1 ml min^{-1}. The mobile phase was filtered through a 0.45 μm membrane-filter (Millipore Co., Bedford, USA) and degassed in a vacuum prior to use.

The calibration curves were constructed by injecting known concentrations of solutions of chlorophyll a and chlorophyll b (Sigma–Aldrich Co., St. Louis, MO, USA) dissolved in butanol. The concentrations were calculated, using the standard calibration curves of chlorophyll a and b.

2.2.4. Determination of pH

The pH values of purees and buffer solutions were determined by using a pH meter (Consort P 407, Schott Gerate, Belgium) calibrated with pH 4.00 and 7.00 buffer. The freshly prepared pea puree had a pH of 6.5.

2.2.5. Calculation of kinetic constant (k)

Degradation rate constants of chlorophylls and visual colour loss were calculated by multiplying the value of the slopes of the regression lines by 2.303. The regression lines were obtained by plotting the logarithms of chlorophylls remaining in peas and colour measurements as a function of blanching times and pH values. The concentrations of chlorophyll a and chlorophyll b as a function of time at a constant temperature for a first-order degradation kinetic model are:

$$\ln(C/C_0) = -kt,$$

where C is the concentration at any time t, C_0 is the initial concentration, and k is the first-order rate constant (min^{-1}).

2.2.6. Calculation of half-life values ($t_{1/2}$)

The half-life values of chlorophyll degradation and colour loss were also calculated using the equation given below:

$$t_{1/2} = \ln 2/k,$$

where k is the rate constant (min^{-1}).

2.2.7. Calculation of activation energy (E_a)

Temperature dependence of chlorophyll degradation and colour loss was determined by the Arrhenius equation:

$$k = k_0 \cdot e^{-E_a/RT},$$

where E_a is the activation energy (kcal mol^{-1}), k is the rate constant, k_0 is the pre-exponential factor, R is the universal gas constant (1.987 kcal mol^{-1}), and T is the absolute temperature (K).

2.2.8. Statistical analyses

All data analyses were performed by using the statistical software programme MINITAB (Release 13.0).

3. Results and discussion

The effect of pH values (5.5, 6.5 and 7.5) on the degradation of chlorophyll a and chlorophyll b in green peas in the 70–100 °C blanching temperature range was investigated. The logarithms of chlorophyll a and chlorophyll b remaining in green peas versus blanching time were plotted for each pH value. A linear relationship was obtained, demonstrating that degradation of chlorophylls a and b followed a first-order kinetic model. The reaction rate constants of chlorophyll a and chlorophyll b are listed in Tables 2 and 3, respectively.

Chlorophyll a and chlorophyll b showed similar trends in different pH conditions. It was observed that the degradation rate of chlorophyll a and chlorophyll b accelerated as pH decreased. The half-life values ($t_{1/2}$) also confirmed

Table 2
Kinetic parameters for chlorophyll *a* degradation in green peas blanching at different pH values

T (°C)	$k \pm$ s.d. (min^{-1})	$t_{1/2} \pm$ s.d. (min)	$E_a \pm$ s.d. (kcal mol^{-1})
pH 5.5			
70	0.0274 ± 0.0016	25.3 ± 1.95	14.0 ± 0.71
80	0.0509 ± 0.0140	13.6 ± 5.73	
90	0.1180 ± 0.0049	5.87 ± 0.35	
100	0.1330 ± 0.0269	5.21 ± 1.56	
pH 6.5			
70	0.0164 ± 0.0004	42.3 ± 1.46	11.7 ± 1.44
80	0.0283 ± 0.0049	24.5 ± 6.19	
90	0.0362 ± 0.0098	19.14 ± 7.91	
100	0.0737 ± 0.0082	9.40 ± 1.49	
pH 7.5			
70	0.0101 ± 0.0013	68.6 ± 11.5	4.80 ± 0.91
80	0.0150 ± 0.0010	46.2 ± 4.38	
90	0.0173 ± 0.0029	40.1 ± 9.77	
100	0.0182 ± 0.0033	38.1 ± 10.1	

s.d., standard deviation for two replicate determinations.

Table 3
Kinetic parameters for chlorophyll *b* degradation in green peas blanching at different pH values

T (°C)	$k \pm$ s.d. (min^{-1})	$t_{1/2} \pm$ s.d. (min)	$E_a \pm$ s.d. (kcal mol^{-1})
pH 5.5			
70	0.0016 ± 0.0001	433 ± 38	10.0 ± 1.22
80	0.0014 ± 0.00	495 ± 0.00	
90	0.0025 ± 0.0002	277 ± 31.6	
100	0.0053 ± 0.0008	131 ± 28.6	
pH 6.5			
70	0.0009 ± 0.0000	770 ± 0.00	11.0 ± 0.24
80	0.0012 ± 0.0001	578 ± 140	
90	0.0014 ± 0.00	495 ± 0.00	
100	0.0039 ± 0.00	178 ± 0.00	
pH 7.5			
70	0.0007 ± 0.0001	990 ± 204	6.84 ± 0.29
80	0.0009 ± 0.00	770 ± 0.00	
90	0.0012 ± 0.0002	578 ± 140	
100	0.0016 ± 0.0001	433 ± 38.4	

s.d., standard deviation for two replicate determinations.

that both chlorophyll *a* and chlorophyll *b* degradations had the fastest rates at the lowest pH. Tables 2 and 3 clearly demonstrate that the half-life values increased as pH increased from 5.5 to 7.5. The rate constants for chlorophyll *a* increased 1.7–3.3 times at applied temperatures with decreasing pH from 6.5 to 5.5. Besides, chlorophyll *a* degraded approximately 2.7–7.3 times faster at pH 5.5 than it did at pH 7.5. Reaction rate constant of chlorophyll *b* degradation increased from 0.0007 to 0.0016 min^{-1} as pH decreased from 7.5 to 5.5 at 70 °C. Similar trends were observed among the rates of chlorophyll *b* degradation at the other blanching temperatures (Table 3). It was found that chlorophyll *a* degraded faster than chlorophyll *b* at 5.5, 6.5 and 7.5 pH for each temperature applied. Many researchers also reported that chlorophyll *a* degraded approximately 2.5 times faster than chlorophyll *b*, regardless of pH (Schwartz & Lorenzo, 1991; Steet & Tong, 1996; Tan & Francis, 1962).

Activation energies were calculated on the basis of linear regression analysis of natural logarithms of rate constants at different pH values against reciprocal absolute temperature, $1/T$ in K (Figs. 1 and 2). When multiplied by 1.987, the slopes of linear regression lines resulted in apparent activation energies of 14.0 ± 0.71, 11.7 ± 1.44 and 4.80 ± 0.91 kcal mol^{-1} for chlorophyll *a* at pH 5.5, 6.5 and 7.5, respectively. While the chlorophyll *a* degradation was affected easily by slight changes in temperature at pH 5.5, the least effect of temperature on chlorophyll *a* degradation was observed at pH 7.5. The higher activation energy at pH 5.5 implies that acidic conditions favour the degradation of chlorophyll *a*.

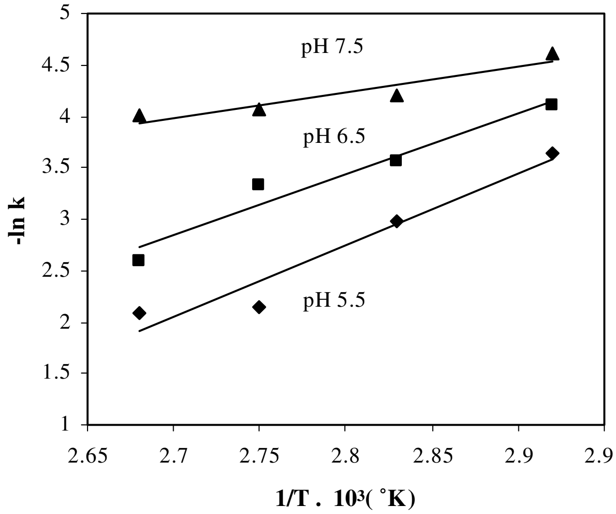

Fig. 1. Arrhenius plots of chlorophyll *a* degradation in green peas blanching under different pH conditions.

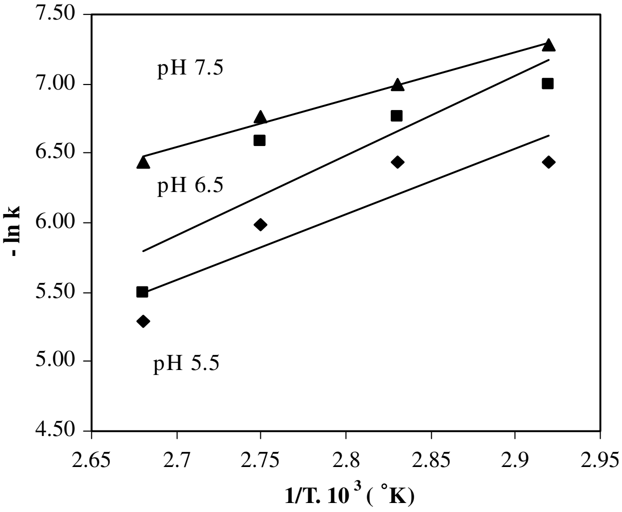

Fig. 2. Arrhenius plots of chlorophyll *b* degradation in green peas blanching under different pH conditions.

Activation energies for chlorophyll *b* at pH 5.5, 6.5 and 7.5 were found to be 10.0 ± 1.22, 11.0 ± 0.24 and 6.84 ± 0.29 kcal mol^{-1}, respectively. Activation energies at pH 6.5, as seen in Tables 2 and 3, indicated that chlorophyll *a*and chlorophyll *b* exhibited almost the same trend in respect of temperature-dependence. The results revealed that chlorophyll *a* was more susceptible to thermal degradation than was chlorophyll *b* in acidic conditions. On the other hand, higher activation energy was determined for chlorophyll *b* degradation at pH 7.5 relative to chlorophyll *a*. Therefore, it is suggested that buffer solution having the highest pH value seems to retain chlorophyll *a* more than chlorophyll *b*. Average activation energies for chlorophyll *a* and chlorophyll *b* were reported to be 14.8 and 15.3 kcal mol^{-1} in the pH range 5.5–7.5 at 80, 90 and 100 °C by Ryan-Stoneham and Tong (2000).

The pH-dependence of chlorophyll degradation in green peas was also determined by using CIE $L^*a^*b^*$ indices. Since the $-a$ value reflects the greenness of sample, the $-a/b$ ratio expresses the conversion of green colour to yellow and h value gives the colour tone, these physical parameters were selected to determine the kinetics of colour loss of peas. Steet and Tong (1996), Weemaes et al. (1999) used the $-a$ value, while Gunawan and Barringer (2000) chose $-a/b$ as the physical property for the determination of kinetic parameters of visual green colour loss.

The green colour loss, on the basis of changes in $-a$, $-a/b$ and h values, as well as the degradation of chlorophyll *a* and *b*, followed a first-order reaction at each pH condition. In addition, significant correlation ($r = 0.9141$–0.9986; $p < 0.05$) between chlorophylls and visual colour parameters ($-a$, $-a/b$ and h values) were found for green peas at each pH-temperature combination studied.

The rate constants of green colour loss decreased with increasing pH, indicating that the green colour was retained at higher pH conditions (Tables 4–6). On the other hand, it seems that the higher pH value (pH 7.5) did not change the rate of colour loss at 70 and 80 °C (Table 6). Therefore, much higher temperatures accelerate green colour losses at higher pH conditions. Gunawan and Barringer (2000) found no significant difference in colour change between pH 7 and pH 8 within the time limits of the experiment. In addition, Sweeney and Martin (1961) determined no further decrease in chlorophyll retention above pH 6.8 and revealed that chlorophyll degradation should occur by the same mechanism at the higher pH but the rate would be so slow that it would require a much longer time frame to study.

Temperature-dependence of visual colour loss was also described by the Arrhenius equation. Since the $-a/b$ value were evaluated as the major indicator for the discoloration of green peas, Arrhenius plots for $-a/b$ value were represented in Fig. 3. The activation energies for $-a$, $-a/b$ and h values were as 8.20 ± 2.26, 9.33 ± 1.70, 8.13 ± 0.71 kcal mol^{-1} at pH 5.5 while those values at pH 7.5 were 10.7 ± 0.87, 8.77 ± 1.34 and 9.01 ± 2.41 kcal mol^{-1}, respectively. As seen in Table 5, activation ener-

Table 4
Kinetic parameters for the change of CIE $L^*a^*b^*$ values in green peas blanching at pH 5.5

	T (°C)	$k \pm$ s.d. (min^{-1})	$t_{1/2} \pm$ s.d. (min)	$E_a \pm$ s.d. (kcal mol^{-1})
$-a$	70	0.0108 ± 0.0006	64.2 ± 5.06	8.20 ± 2.26
	80	0.0124 ± 0.0015	55.9 ± 9.70	
	90	0.0216 ± 0.0037	32.1 ± 8.01	
	100	0.0269 ± 0.0067	25.8 ± 9.67	
$-a/b$	70	0.0092 ± 0.0001	75.3 ± 1.15	9.33 ± 1.70
	80	0.0140 ± 0.0016	49.5 ± 8.11	
	90	0.0212 ± 0.0035	32.7 ± 7.84	
	100	0.0279 ± 0.0042	24.8 ± 5.41	
h	70	0.0021 ± 0.0002	330 ± 44.9	8.13 ± 0.71
	80	0.0028 ± 0.0001	248 ± 12.5	
	90	0.0039 ± 0.00	178 ± 0.00	
	100	0.0055 ± 0.0010	126 ± 33.50	

s.d., standard deviation for two replicate determinations.

Table 5
Kinetic parameters for the change of CIE $L^*a^*b^*$ values in green peas blanching at pH 6.5

	T (°C)	$k \pm$ s.d. (min^{-1})	$t_{1/2} \pm$ s.d. (min)	$E_a \pm$ s.d. (kcal mol^{-1})
$-a$	70	0.0039 ± 0.0003	178 ± 19.5	11.90 ± 0.88
	80	0.0076 ± 0.0015	91.2 ± 26.5	
	90	0.0097 ± 0.00	71.4 ± 0.00	
	100	0.0180 ± 0.0010	38.5 ± 3.04	
$-a/b$	70	0.0046 ± 0.0010	151 ± 48.6	12.0 ± 1.07
	80	0.0076 ± 0.0011	91.2 ± 19.1	
	90	0.0117 ± 0.0013	59.2 ± 9.42	
	100	0.0191 ± 0.0045	36.3 ± 12.8	
h	70	0.0009 ± 0.0004	770 ± 123	11.7 ± 0.73
	80	0.0016 ± 0.0001	433 ± 38.4	
	90	0.0023 ± 0.00	301 ± 0.00	
	100	0.0039 ± 0.0008	178 ± 53.8	

s.d., standard deviation for two replicate determinations.

Table 6
Kinetic parameters for the change of CIE $L^*a^*b^*$ values in green peas blanching at pH 7.5

	T (°C)	$k \pm$ s.d. (min^{-1})	$t_{1/2} \pm$ s.d. (min)	$E_a \pm$ s.d. (kcal mol^{-1})
$-a$	70	0.0018 ± 0.0005	385 ± 164	10.7 ± 0.87
	80	0.0018 ± 0.0002	385 ± 61.3	
	90	0.0041 ± 0.0005	169 ± 29.6	
	100	0.0060 ± 0.0006	116 ± 16.5	
$-a/b$	70	0.0025 ± 0.00	277 ± 0.00	8.77 ± 1.34
	80	0.0023 ± 0.0003	301 ± 56.5	
	90	0.0055 ± 0.0001	126 ± 3.24	
	100	0.0062 ± 0.0010	118 ± 26.2	
h	70	0.0005 ± 0.0001	1386 ± 408	9.01 ± 2.41
	80	0.0005 ± 0.00	1386 ± 0.00	
	90	0.0012 ± 0.00	578 ± 0.00	
	100	0.0012 ± 0.00	578 ± 0.00	

s.d., standard deviation for two replicate determinations.

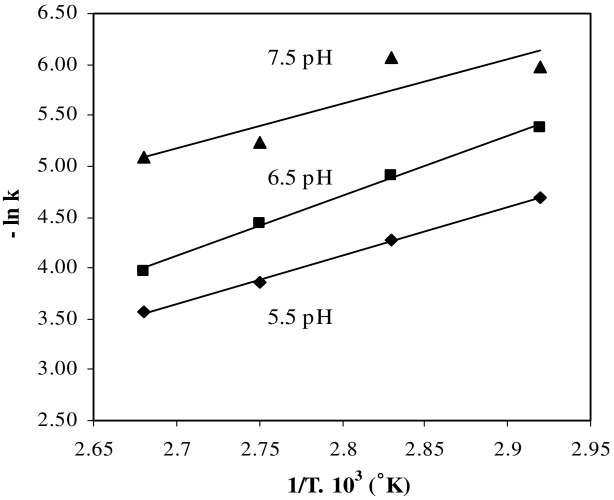

Fig. 3. Arrhenius plots for the changes in −*a*/*b* values of green peas under different pH conditions.

gies for those colour parameters at pH 6.5 were slightly higher than at pH 5.5 and 7.5.

The effect of pH on chlorophyll degradation and colour loss in different vegetables was also investigated by other researchers. Lajollo and Marquez (1982) studied the degradation of chlorophyll in spinach at low and intermediate water activities at four different pH conditions and found that the degradation obeyed a first-order model. Gunawan and Barringer (2000) also studied the effect of pH on the rate of colour change in broccoli at pH 3–8 by using HPLC and showed that pheophytinization followed a first-order reaction and colour degradation accelerated with decreasing pH, as expected.

4. Conclusion

The first-order reaction kinetics were determined for chlorophyll degradation and visual colour loss in blanched green peas in buffered solutions of pH 5.5, 6.5 and 7.5 in the temperature range 70–100 °C. The rate constants of green colour loss and chlorophyll degradation decreased with increasing pH, indicating that the green colour was retained at higher pH conditions. It was found that chlorophyll *a* degraded faster than chlorophyll *b* at all pH values for each temperature applied. The results revealed that chlorophyll *a* was more susceptible to thermal degradation than chlorophyll *b* in acidic conditions. The significant relationship ($r = 0.9301$–0.9986; $p < 0.05$) found between the change of visual colour parameters (−*a*, −*a*/*b* and *h* values) and degradation of chlorophyll *a*, as well as chlorophyll *b* ($r = 0.9141$–0.9984; $p < 0.05$), indicated that the objective colour measurements can be used for determining the degradation kinetics of chlorophyll *a* and *b* instead of expensive and time-consuming HPLC analysis.

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