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EFFECT OF HEAT TREATMENT ON CHLOROPHYLL DEGRADATION AND COLOR LOSS IN GREEN PEAS

BEZELYELERDE KLOROFİL DEGRADASYONU VE RENK KAYBI ÜZERİNE ISIL İŞLEMİN ETKİSİ

Hande Selen ERGE, Feryal KARADENİZ*, Nuray KOCA, Yeşim SOYER
Ankara Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, Ankara

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ABSTRACT : Thermal degradation kinetics of chlorophyll and visual green color loss in green peas were investigated at 70°, 80°, 90° and 100 °C. The degradation of chlorophyll *a* and chlorophyll *b* followed a first-order kinetic model. The loss of visual green color, as represented by the change of the -a, the ratio -a/b and h (hue) values measured by tristimulus colorimeter, also followed a first-order reaction. The activation energies for chlorophylls *a* and *b* were determined as 47.78 and 26.77 kJ mol^{-1}, while those for -a, -a/b and h values were found as 49.75, 56.04 and 55.06 kJ mol^{-1}, respectively.

Keywords: Chlorophyll, kinetics, green peas, color

ÖZET : Bezelyelerde klorofilin termal degradasyon kinetiği ve görünür yeşil renkteki kayıp 70°, 80°, 90° ve 100 °C'de araştırılmıştır. Klorofil *a* ve klorofil *b*'nin parçalanması birinci dereceden bir kinetik model izlemektedir. Tristimulus kolorimetresi ile ölçülen -a, -a/b ve h (hue) değerlerindeki değişim ile ortaya konulan görünür yeşil renkteki kayıp da birinci dereceden reaksiyona uymaktadır. Klorofil *a* ve *b* için aktivasyon enerjileri sırasıyla 47.78 and 26.77 kJ mol^{-1} olarak belirlenirken; -a, -a/b ve h değerleri için aktivasyon enerjileri 49.75, 56.04 and 55.06 kJ mol^{-1} olarak saptanmıştır.

Anahtar kelimeler: Klorofil, kinetik, bezelye, renk

INTRODUCTION

Chlorophylls are the most widely distributed plant pigments responsible for the characteristic green color of fruit and vegetables (1). The major chlorophylls in foods are chlorophyll *a*, which has a methyl group at C-3 carbon, and chlorophyll *b*, which a formyl group is bonded to the same carbon atom. 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 (2), as well as their thermal stability. Chlorophyll *a* was reported to be thermally less stable than chlorophyll *b* (3, 4, 5, 6, 7).

Chlorophylls are known to be easily degraded by conditions such as dilute acids, heat, light and oxygen (8). Since color is a major sensory characteristic in determining product acceptibility, it is important to prevent or at least minimize chlorophyll degradation during thermal processing in food industry. Thermal processing induces structural and chemical variations to the tissue of vegetables that often result in color changes (6, 9, 10). The reason for the green color loss during processing is mainly attributed to the conversion of chlorophylls to pheophytins by the influence of pH (11). In acidic medium, magnesium in the chlorophyll ring is replaced by two hydrogen ions and green chlorophylls are converted to the olive brown pheophytins (12, 13, 14). Formation of pheophytins during heating is initiated by the release of cellular acids and the synthesis of new acids. It is reported that formation of pheophytin in processed vegetables is increased at lower tissue pH values and at hig-

* **E-mail :** karadeni@eng.ankara.edu.tr

her process temperatures (15). The formation of derivatives known as pyropheophytins may also occur by the loss of the carbomethoxy group from pheophytins (5, 12) as a result of further heating (6, 12). Similar to pheophytins, pheophorbides also may occur under the influence of heat or acid (16). The cleavage of the phytol chain of chlorophyll by the enzyme chlorophyllase results in the formation of chlorophyllide (10). In the presence of acid, chlorophyllides undergo loss of the magnesium and form phephorbides (10, 17). Chlorophyllase also hydrolyzes the phytol chain of pheophytins giving rise to pheophorbides (10). It is reported that this enzyme acts at ambient temperature only in the presence of high concentrations of organic solvents and only at temperatures between 65° and 75 °C in aqueus medium (11). On the other hand, chlorophyllase losses its activity when heated to 100 °C. Since the optimum temperature for chlorophyllase activity in vegetables ranges between 60 °C and 82.2 °C, some green vegetables show considerable formation of chlorophyllides and some pheophorbides only at low-temperature (60-70 °C) blanching treatment (18, 19, 20).

Practically, strong thermal treatment has been used to achieve commercial sterility of vegetables. Therefore, the kinetic parameters including reaction order, rate constant and activation energy are essential to predict the quality loss during thermal processing. 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. 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 (21, 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 (16, 24). Additionally, some other researchers (25) have monitored changes in chlorophyll content by the ratio of -a to b value (-a/b) for canned green peas and green beans.

The objectives of this study were to determine the degradation kinetics of chlorophyll *a* and chlorophyll *b* by using HPLC and visual green color using CIE-L*a*b* color system in thermally treated green peas. In addition, the correlation of the degradation of chlorophyll *a* and *b* with the change in visual green color was compared.

MATERIALS AND METHODS

Chemicals

HPLC grade solvents methanol, ethylacetate, buthanol and acetone were obtained from Merck (Darmstadt, Germany); while chlorophyll *a* and chlorophyll *b* standards from spinach were purchased from Sigma-Aldrich Co. (St. Louis, MO., USA).

Materials

Fresh green peas (*Pisum sativum*, L. var. Bolero) were supplied by one of the biggest canning company in Turkey and stored at 0 °C under 95 % relative humidity in polyethylene bags until the analyses. All analyses were completed within a week.

Blanching treatment

Dehulled green peas were put in cheese-cloth bags and applied to heat treatment at 70° (10, 20, 30, 40, 50, 60, 70 min.), 80° (10, 15, 25, 35, 45, 55 min.) and 90 °C (5, 10, 15, 20, 30, 40 min.) in a water-bath (Memmert, Schwabach, Germany) and at 100°C (3, 6, 9, 12, 15, 20, 25 min.) in an oil-bath (Normschliff, Wertheim). At the end of heating periods, samples were taken and immediately cooled under the tap water at 22 °C. Blanched peas were allowed to drain and mashed in a mortar before conducting following methods of analyses. Blanching treatments were carried out with two replicates at selected time-temperature combinations.

Methods

Determination of color values

The -a, b and h values for each green pea sample were measured by a tristimulus colorimeter (Model CR-300, Minolta Chromameter, Osaka, Japan). The data on -a and b values were also used to calculate the -a/b ratio.

Extraction

Pigment extraction from pea puree was conducted by following the method of Canjura et al. (6) in two replications. For this purpose, 18.8 mL of acetone was added to 5g of sample and the mixture was homogenized with Ultra-Turrax homogenizer (IKA Werke, Labortechnik, Staufen, Germany) for 2 minutes. The slurry was then filtered under vacuum through Whatman 42 filter paper. The filtrate was brought to volume with 80 % acetone in a 25 mL volumetric flask. The extract was filtered through a 0.45 mm durapore membrane filter (HVHP Millipore Co., Watford, Ireland). 20 µL of filtered sample was immediately injected onto the HPLC.

HPLC analyses

HPLC separation of chlorophyll pigments were achieved on a C-18 column, 5 µ particles, 4.6 mm.x 25 cm i.d (Zorbax, USA) with a Zorbax, ODS analytical guard column (5µ particle size, 4.6 x 12.5 mm i.d) according to a modified method of Schwartz and von Elbe (5). Chlorophyll *a* and chlorophyll *b* were separated by using an isocratic mobil phase of ethylacetate: methanol: water (50: 37.5: 12.5) at a flow rate of 1 mL min^{-1} supplied by the Waters 510 HPLC pump (Millipore Co., Milford). The chlorophyll pigments were monitored at 430 nm using Waters 486 UV-VIS detector (Millipore Co., Milford). The concentrations were calculated using the standard calibration curves of chlorophyll *a* and *b*. The calibration curves were constructed by injecting known concentrations of solutions of chlorophyll *a* and chlorophyll *b* dissolved in buthanol. Identification of peaks was confirmed by comparing retention times obtained from chlorophyll *a* and chlorophyll *b* standarts. The retention time for chlorophyll *a* was 27 min, while that for chlorophyll *b* was 16 min. Integration and data storage were performed with Millenium 2010 chromatography software (Millipore Co., Milford).

Calculation of kinetic parameters

Rate constants of chlorophyll degradation and visual color loss were calculated by multiplying the value of the slopes of the regression lines by 2.303 (26). The regression lines were obtained by plotting the logarithms of color values (-a, -a/b and h) and log percent of chlorophylls remaining in green peas as a function of blanching times. A first-order model to describe the degradation of chlorophyll in green peas is given below:

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

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

The temperature dependence of chlorophyll degradation and color loss was modelled with the Arrhenius equation (27):

$$k = k_0 . e^{-Ea/RT}$$

where k: rate constant; k_0 : pre-exponential factor; E_a: activation energy (kJ mol^{-1}); R: gas constant (8.314 x 10^{-3} kJ mol^{-1} K^{-1}); T: temperature in K

Temperature quotients (Q_{10}) were calculated using the equation given below:

$$Q_{10} = (k_2 / k_1)^{10/T_2 - T_1}$$

where k_1: rate constant at T_1; k_2: rate constant at T_2; T_1, T_2: absolute temperatures (K)

Half-life value ($t_{1/2}$), the time needed for 50 % degradation for chlorophyll *a* and chlorophyll *b*, was calculated following the equation:

$t_{1/2} = - \ln 0.5 / k$

where k: rate constant

RESULTS AND DISCUSSION

Thermal degradation of chlorophylls *a* and *b* in green peas was studied in the temperature range of 70-100 °C. The degradation of chlorophylls *a* and *b* in green peas followed a first-order reaction kinetic model (Figures 1 and 2) which is consistent with the reported data (2, 5, 6, 25, 28). Table 1 lists the reaction rate costants and the determination coefficients for the degradation of chlorophylls at all temperatures studied. Chlorophyll *a* degraded 12 to 18 times faster than chlorophyll *b* depending on temperature, indicating that chlorophyll *a* is more susceptible to thermal treatments.

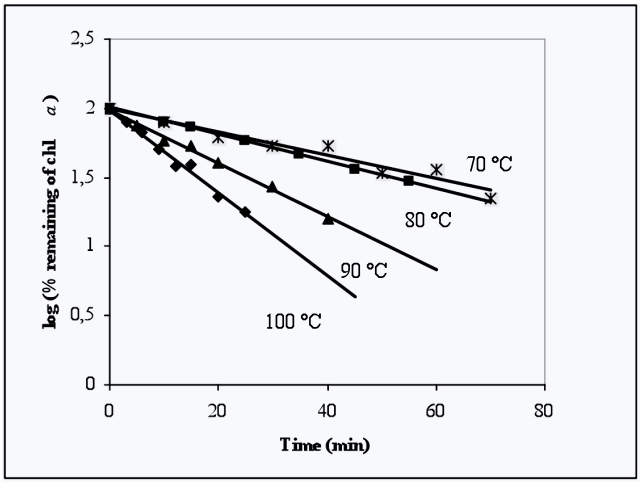

Figure 1. Changes in chlorophyll *a* content of green peas during heating at various temperatures

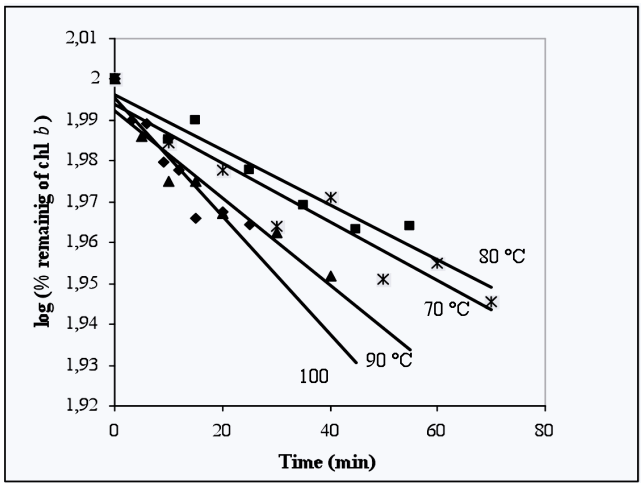

Figure 2. Changes in chlorophyll *b* content of green peas during heating at various temperatures

Table 1. Kinetic parameters of first-order chlorophyll degradation and visual color loss in green peas

	Temp (°C)	k (min-1)	$t_{1/2}$ (min)	Q_{10}			E_a (kJ mol^{-1})
				70-80 °C	80-90 °C	90-100 °C	
Chlorophyll a	70	-0.0193 (0.9478)1	35.91	1.16	1.98	1.57	47.78
	80	-0.0223 (*0.9977*)	31.08				
	90	-0.0442 (*0.9937*)	15.68				
	100	-0.0693 (*0.9859*)	10.00				
Chlorophyll *b*	70	-0.0016 (*0.9085*)	433.22	1.00	1.57	1.55	26.77
	80	-0.0016 (*0.9219*)	433.22				
	90	-0.0025 (*0.9130*)	277.26				
	100	-0.0039 (*0.8985*)	177.73				
-a	70	-0.0062 (*0.9363*)	111.80	1.15	1.94	1.85	49.75
	80	-0.0071 (*0.9538*)	97.63				
	90	-0.0138 (*0.9726*)	50.23				
	100	-0.0256 (*0.9628*)	27.08				
-a/b	70	-0.0048 (*0.9900*)	144.41	1.62	1.53	2.23	56.04
	80	-0.0078 (*0.9565*)	88.87				
	90	-0.0120 (*0.9525*)	57.76				
	100	-0.0267 (*0.9666*)	25.96				
h	70	-0.0009 (*0.9908*)	753.42	1.75	1.43	2.20	55.06
	80	-0.0016 (*0.9484*)	433.22				
	90	-0.0023 (*0.9391*)	300.98				
	100	-0.0051 (*0.9506*)	136.99				

1: Determination coefficients of first order reaction for chloropyll degradation and color loss in green peas

Activation energies were calculated on the basis of linear regression analysis of natural logarithms of rate constants against reciprocal absolute temperature, 1/T in K (Figure 3). When multiplied by 8.314, the slopes of linear regression lines resulted in apparent activation energies of 47.78 and 26.77 kJ mol^{-1} for chlorophyll *a* and chlorophyll *b*, respectively. This result was consistent with the previous values which indicated the activation energy of the *b* form was less than that of the *a* form (5, 29). The higher activation energy implies that a smaller temperature change is sufficient to degrade chlorophyll *a* more rapidly. A wide range of activation energies, from 52 to 105 kJ mol^{-1} for chlorophyll *a* and from 31 to 94 kJ mol^{-1} for chlorophyll *b*, for degradation of chlorophylls in spinach puree have been reported in previous studies (5, 29, 30) .

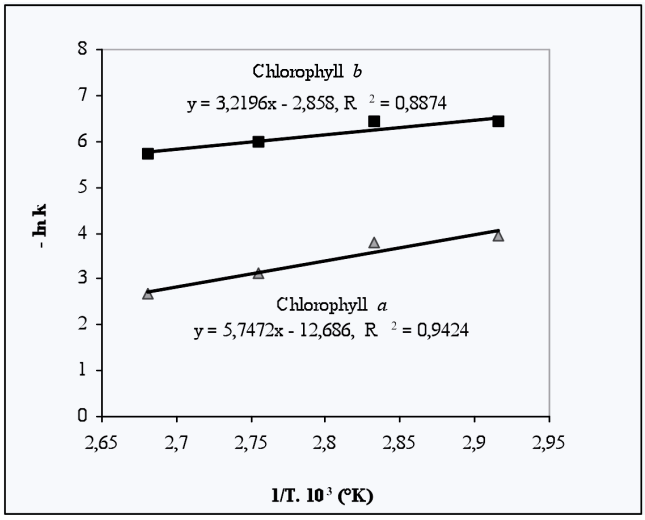

Figure 3. Arrhenius plots of chlorophyll degradation in green peas

Degradation of chlorophyll *a* at 100 °C had the fastest rate with a half-life ($t_{1/2}$) value of 10 min, followed by 90°, 80° and 70 °C which had $t_{1/2}$ values of 15.68, 31.08 and 35.91 min, respectively (Table 1). On the other hand, $t_{1/2}$ values for the chlorophyll *b* degradation at applied temperatures were found to be higher than those of chlorophyll *a* degradation.

Kinetic parameters for the thermal degradation of chlorophyll were also determined on the basis of visual color changes 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 color to yellow and h value gives the color tone, these physical parameters were selected to determine the kinetics of color loss of green peas. In the range of applied temperatures, when the logarithms of the color parameters were plotted against heating times, the relationship was linear indicating that the degradation of visual green color followed a first-order reaction. Figure 4 shows the first-order kinetic reaction for the change of -a values. As can be seen from the rate constants, visual green color (-a) and h values of green peas decreased as a function of heating time at 70°, 80°, 90° and 100 °C (Table 1). The reduction of h values indicates the color of green pea changes to yellow after heat treatment. Steet and Tong (2) also reported that visual green color loss in pureed green peas followed a first-order reaction by using –a value as a physical property.

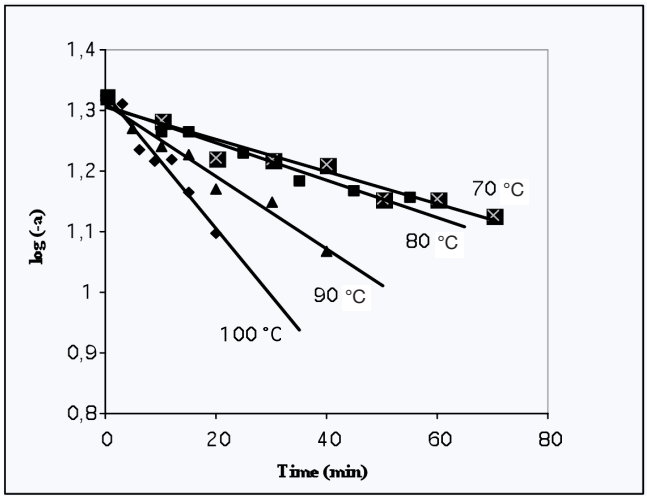

Figure 4. Changes of -a values in green peas

Figure 5 shows the Arrhenius plots describing the temperature-dependence of color changes in green peas. Values for the apparent first-order reaction rate constant, half-life value ($t_{1/2}$) and temperature quotients (Q_{10}) are presented in Table 1. Activation energies determined on the basis of CIE L*a*b* parameters (-a, -a/b, h) were 49.75, 56.04 and 55.06 kJ mol^{-1}, respectively. Hayakawa and Timbers (28) also used -a/b value for determining visual color changes and activation energies for green color loss in asparagus, green beans and green peas were found as 75.7, 82.8 and 63.6 kJ mol^{-1}, respectively. In additon, an activation energy of 76.2 kJ mol^{-1} has been determined by Steet and Tong (2) for pureed green peas when -a value is taken into consideration.

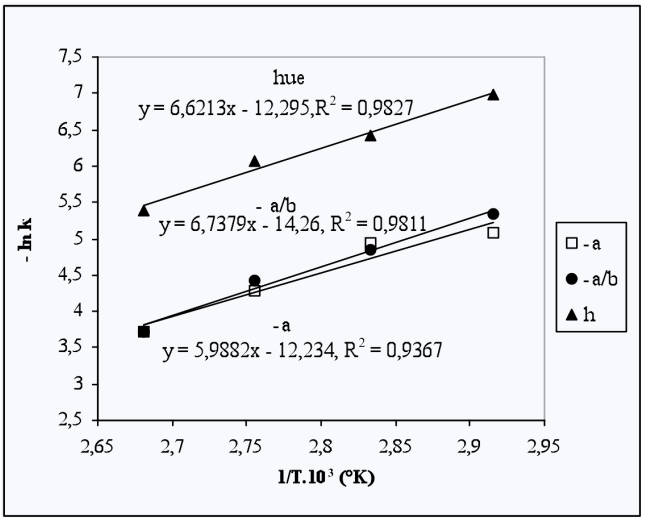

Figure 5. Arrhenius plots for the changes in CIE-L*a*b * values of green peas

The activation energies for changes in visual green color and that for the degradation of chlorophyll *a* in green peas were found to be similar. On the other hand, the activation energy for chlorophyll *b* was less than that of visual color loss. The green color of peas is composed of chlorophyll *a* responsible for blue-green color of vegetables 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 peas approaches to yellow, gradually. The physical parameters (-a, -a/b and h) also decrease as a result of the loss of green color intensity.

CONCLUSION

The first-order reaction kinetics was determined for color degradation in green peas by using high performance liquid chromatography and tristimulus colorimetry. The significant relationship ($r = 0.967$-0.978, $p < 0.01$) was found between the change of visual color parameters and degradation of chlorophyll *a* as well as chlorophyll *b* ($r = 0.938$-0.989, $p < 0.01$). Since the activation energies for visual green color loss and chlorophyll *a* degradation were found similar, it is suggested that the objective color measurements can be used for determining the degradation kinetics of chlorophyll *a* instead of expensive and time consuming HPLC analysis.

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