Kinetics of Chlorophyll Degradation and Color Loss in Heated Broccoli Juice

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Degradation of chlorophyll in broccoli juice occurred at temperatures exceeding 60 °C. Chemical analysis revealed that degradation of chlorophyll *a* and *b* to pheophytin *a* and *b*, respectively, followed first-order kinetics and that chlorophyll *a* was more heat sensitive than chlorophyll *b*. Temperature dependencies of chlorophyll *a* and *b* degradation rate constants could be described by Arrhenius equations with activation energies (E_a) of 71.04 ± 4.89 and 67.11 ± 6.82 kJ/mol, respectively. Objective greenness measurements, using the $-a$ value as the physical property, together with a fractional conversion kinetic analysis, indicated that green color degradation followed a two-step process. Kinetic parameters for the first degradation step were in accordance with the kinetic parameters for pheophytinization of the total chlorophyll content, as determined by chemical analysis ($E_a \approx 69$ kJ/mol). The second degradation step, that is, the subsequent decomposition of pheophytins, was characterized by an activation energy of 105.49 ± 4.74 kJ/mol.

Keywords: *Chlorophyll; pheophytin; green color; broccoli (Brassica oleracea L. Italica); thermal degradation*

INTRODUCTION

Chlorophyll *a* and *b* pigments are typically found in higher plants (in an approximate ratio of 3:1) and are responsible for the green color of many vegetables (Schwartz and Lorenzo, 1990). In intact plant tissue, chlorophyll degrades enzymatically as well as chemically (by heat or acid). Cleavage of phytol from chlorophyll (or its magnesium-free derivative pheophytin) by the membrane-bound enzyme chlorophyllase, hereby forming chlorophyllide (or pheophorbide), does not occur until the enzyme has been activated postharvest (e.g., by mild heat treatments). The chlorophyllase activity is, however, seriously reduced upon heating above 80 °C (von Elbe and Schwartz, 1996). During thermal processing (or acidification), the central magnesium atom of the porphyrin ring of chlorophyll is replaced by two hydrogen atoms to form pheophytin, which is accompanied by an undesirable color change from bright green to olive brown (Mackinney and Joslyn, 1941; Gold and Weckel, 1959; Schwartz and Lorenzo, 1990). Similarly, enzymatically generated chlorophyllide converts to pheophorbide under the influence of heat (or acid).

Since color is a major, if not the most important, sensory characteristic in determining product acceptability, it is of critical importance to the food industry to prevent or at least minimize chlorophyll degradation during (thermal) processing (Villota and Hawkes, 1986; von Elbe and Schwartz, 1996). For such optimization purposes, it is of utmost importance to determine kinetic parameters (reaction orders, rate constants, activation energies) for chlorophyll degradation (and other organoleptic characteristics), as well as for microbial destruction under the influence of heat.

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In the present study, kinetic analyses of thermal degradation of organoleptic characteristics in a food product, namely broccoli juice, are focused upon. Hereto, the following steps were taken. Initially, thermal degradation of chlorophyll *a* and *b* was studied by means of spectrophotometrical determination of their concentrations remaining after numerous temperature−time combinations. Thereafter, attention was paid to the color loss of the juice upon heating; that is, the changes in *L*, *a*, and *b* color values were monitored as a function of heating time. Finally, correlation of the degradation of chlorophyll with the change in broccoli greenness was attempted ($\sim -a$ color value).

MATERIALS AND METHODS

Sample Preparation. Broccoli (*Brassica oleracea* L. *Italica*) was purchased from a local auction. Broccoli florets were squeezed (liquidizer, Braun, Germany, type MP 32) and the batch of broccoli juice obtained (pH 6.65) was stored under liquid nitrogen in 50 mL screw cap tubes (Greiner, Frickenhausen, Germany) until used. Chlorophyllase was not extracted using this procedure so that none of the conversions of chlorophyll or its derivatives during the subsequent heat treatments were enzyme catalyzed.

Before the thermal degradation experiments were carried out, the samples were thawed overnight, protected from light in a refrigerator at 7 °C, and subsequently filtered.

Thermal Treatment. Kinetic parameter values for thermal degradation of chlorophyll and (green) color were determined on the basis of isothermal heating experiments and determination of the residual chlorophyll content or postprocessing *L a b* color values. Hereto, Chrompack crimp top vials (i.d. 8 mm, 800 μL, Middelburg, The Netherlands) filled with broccoli juice were heated in a thermostated oil bath (Grant, Cambridge, England) during preset times (eight vials per heating time). After withdrawal from the oil bath, the samples were immediately transferred to ice water to avoid cooling lags.

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Pigment Extraction. In a darkened room, chlorophyll was extracted from broccoli juice using 80% (v/v) acetone. After 15 min, the solution was filtered (refined cellulose filter paper, 4−7 μm pore size, Schleicher and Schuell, 595, Dassel, Germany) and centrifuged (Beckman J2-HS centrifuge, Palo Alto, CA; 12100*g*, 10 min).

Determination of Chlorophyll Concentration. The chlorophyll concentration was determined as described by Vernon (1960). Saturated oxalic acid [2% (v/v)] in 80% (v/v) acetone was added to half of the pigment solution to convert chlorophyll to pheophytin. Both acidified and nonacidified pigment solutions were stored at room temperature, protected from light, for exactly 3 h. Subsequently, absorbance readings of the acidified and nonacidified pigment solutions were made at both 662 and 645 nm (Ultrospec K, Pharmacia, LKB, Biochrom Ltd., Cambridge, England). The concentrations of chlorophyll *a* and *b* and the total chlorophyll content were calculated using conversion formulas 1, 2, and 3, which take

$$[\text{chlorophyll } a] = 25.38\Delta A_{662} + 3.64\Delta A_{645} \quad (1)$$

$$[\text{chlorophyll } b] = 30.38\Delta A_{645} - 6.58\Delta A_{662} \quad (2)$$

$$[\text{chlorophyll total}] = 18.80\Delta A_{662} + 34.02\Delta A_{645} \quad (3)$$

into account the possible presence of pheophytin *a* and *b* in the extracted broccoli juice. ΔA_{662} symbolizes the absorbance difference of the nonacidified and acidified pigment solution at 662 nm and ΔA_{645} the absorbance difference of the nonacidified and acidified solution at 645 nm. Absorbance readings were made in duplicate.

Determination of *L*, *a*, and *b* Color Values. Objective color measurements were made with a Colorquest 45/0 spectrophotometer (Hunterlab, Reston, VA). The apparatus (45°/0° geometry, illuminant D65, 10° observer) was calibrated with a standard white tile ($X = 78.66$, $Y = 83.31$, $Z = 88.40$). A cylindrical glass cell filled with 2 mL of broccoli juice was placed on top of the light source (2.5 cm opening) and covered with a white plate. Inclusion of air bubbles was hereby avoided. The recorded tristimulus values *X*, *Y*, and *Z* were converted to *L*, *a*, and *b* color values by a connected computer. Color measurements were carried out in duplicate.

Data Analysis. Chlorophyll degradation data obtained from the chemical analysis, that is, the spectrophotometrical method described by Vernon (1960), were conventionally analyzed assuming first-order degradation kinetics (eq 4). Chlorophyll

$$\ln\left(\frac{C}{C_0}\right) = -kt \quad (4)$$

degradation has indeed frequently been reported to exhibit first-order kinetics (Canjura et al., 1991; Steet and Tong, 1996). First-order degradation kinetics of chlorophyll in broccoli have been confirmed in the literature (Van Loey et al., 1998). Degradation rate constants (*k*) were derived from the slopes of the regression lines obtained when plotting $\ln(C/C_0)$ as a function of heating time.

Since color is a physical property, the color degradation kinetics were analyzed using a fractional conversion technique, as suggested by Steet and Tong (1996). For an irreversible first-order degradation reaction, the fractional conversion concept can be written as

$$P = P_\infty + (P_0 - P_\infty)e^{-kt} \quad (5)$$

with *P* symbolizing the physical parameter under consideration, that is, the *L*, *a*, or *b* color value.

Equation 5 was used to estimate *k* (and P_∞) for the different degradation temperatures studied, using nonlinear regression analysis (*SAS/STAT User's Guide*, 1994).

In all cases, temperature dependence of the degradation rate constant could adequately be described by the Arrhenius equation:

$$\ln(k) = \ln(k_{\text{ref}}) - \left(\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)\right) \quad (6)$$

Activation energies were estimated on the basis of linear regression analysis of $\ln(k)$ vs reciprocal absolute temperature ($1/T$).

RESULTS AND DISCUSSION

Chemical analysis of chlorophyll *a* and *b* in untreated broccoli juice revealed that the chlorophyll *a* to chlorophyll *b* ratio equaled 2.9:1 on average. This value is identical to the ratio obtained by Van Loey et al. (1998) for broccoli but is slightly higher than the values of 2.4 and 2.03 reported by Vernon (1960) and Sweeney and Martin (1958), respectively.

Thermal degradation of chlorophyll *a* and *b* to pheophytin *a* and *b*, respectively, was studied in the 80−120 °C temperature range. The increase in pheophytin content, as measured by the procedure described by Vernon (1960), upon processing at 80 °C is shown in Figure 1. The heating time was measured from the time the product temperature reached the processing temperature. The pheophytin content at heating time 0 is thus due to pheophytinization during pigment extraction and the time interval needed to reach the desired processing temperature.

Both chlorophyll *a* and chlorophyll *b* degradation appeared to follow first-order degradation kinetics (Figure 2). Correlation coefficients for the linear regression analysis were situated between 0.964 and 0.988 for chlorophyll *a* and between 0.925 and 0.987 for chlorophyll *b* degradation. Table 1 summarizes the degradation rate constants for chlorophyll *a* and *b*, as well as for the total chlorophyll content. For the latter, correlation coefficients for the linear regression ranged from 0.964 to 0.993. From Table 1 it is clear that chlorophyll *a* is more heat sensitive than chlorophyll *b*, a feature that was also observed for degradation of chlorophyll in other food products (Lund, 1975). The higher thermal stability of chlorophyll *b* has been attributed to the electron-withdrawing effect of its C-3 formyl group (von Elbe and Schwartz, 1996). In agreement with the study of Schwartz and von Elbe (1983) on thermal chlorophyll degradation in spinach, chlorophyll *a* degraded at a rate approximately twice that of chlorophyll *b*. It has, however, to be mentioned that the estimated degradation rate constants deviate from those reported by Van Loey et al. (1998). Variation in the overall composition (including pH) of the broccoli juice tested might account for the divergent degradation rate constants. Even subtle differences in the molecular environment seem to influence the rate of chlorophyll degradation in natural systems (Haisman and Clarke, 1975).

Temperature dependence of the degradation rate constants could adequately be described by the Arrhenius equation (Figure 3). Corresponding activation energies equaled about 71, 67, and 69 kJ/mol for chlorophyll *a*, chlorophyll *b*, and total chlorophyll content, respectively (Table 2). Correlation coefficients for the linear regression analysis equaled 0.986, 0.970, and 0.986, respectively. The temperature coefficients for chlorophyll *a* and *b* are respectively higher and lower than the values reported by Van Loey et al. (1998), and their ranking is reversed. However, based on their 95% confidence intervals, the activation energies obtained in both studies were not found to be significantly different.

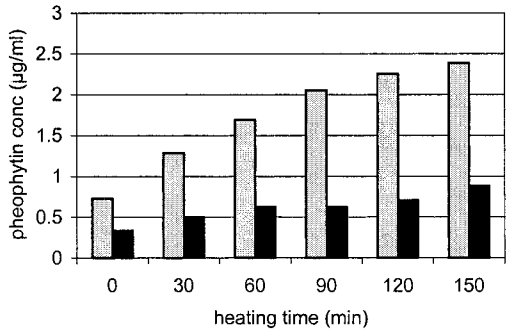

Figure 1. Increase in pheophytin *a* (gray bars) and *b* (black bars) content upon thermal processing at 80 °C.

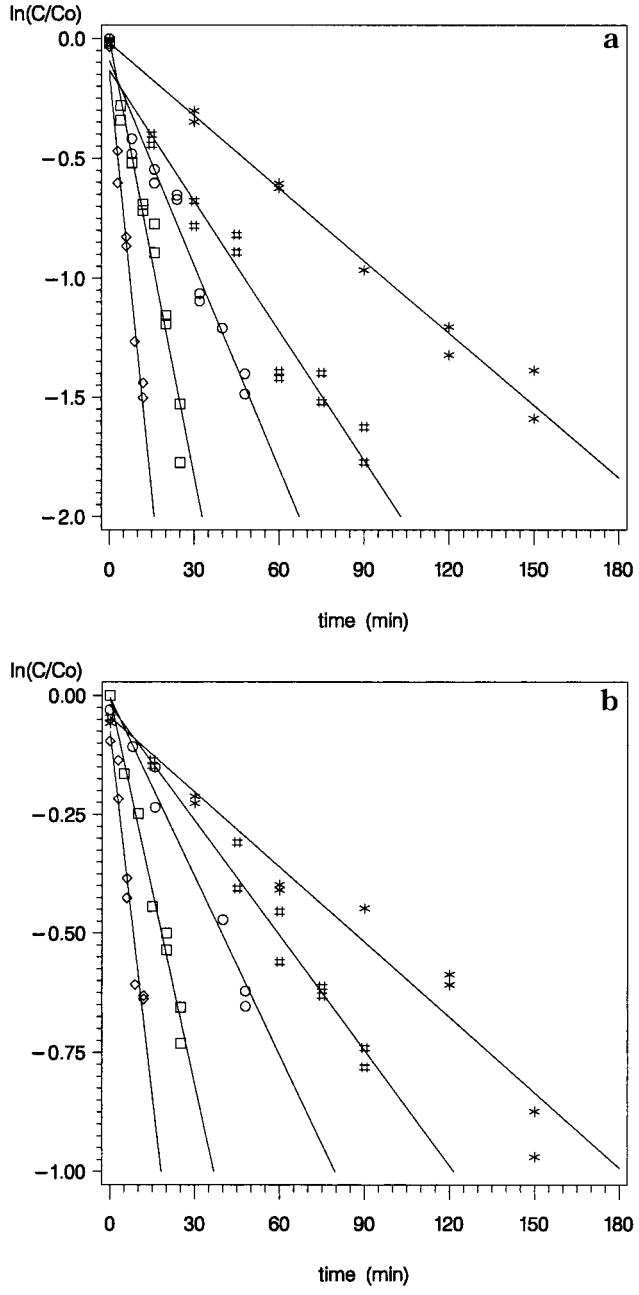

Figure 2. First-order thermal degradation of (a) chlorophyll *a* and (b) chlorophyll *b* in broccoli juice at (*) 80, (#) 90, (○) 100, (□) 110, and (◇) 120 °C.

In a subsequent study, the green color loss of broccoli juice upon heating was studied in depth for temperatures ranging from 60 to 120 °C. For the kinetic analysis

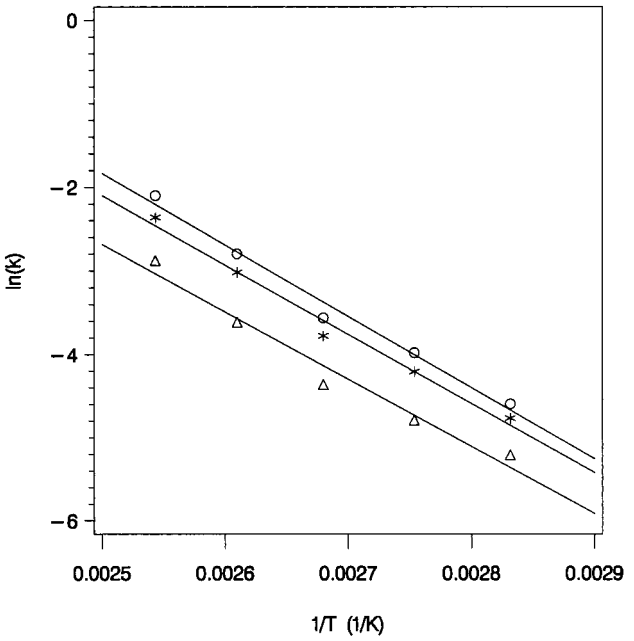

Figure 3. Variation with temperature of the degradation rate constants: (○) chlorophyll *a*, (△) chlorophyll *b*, and (*) total chlorophyll.

Table 1. Rate Constants ($\times 10^2$; min^{-1}) for Thermal Degradation of Chlorophyll in Broccoli Juice, Determined by Chemical Analysis

temp (°C)	chlorophyll *a*	chlorophyll *b*	total chlorophyll
80	1.01 ± 0.04[a]	0.55 ± 0.04[a]	0.85 ± 0.02[a]
90	1.87 ± 0.10	0.83 ± 0.04	1.49 ± 0.07
100	2.84 ± 0.15	1.28 ± 0.07	2.29 ± 0.10
110	6.11 ± 0.34	2.70 ± 0.12	4.89 ± 0.15
120	12.24 ± 0.70	5.64 ± 0.57	9.43 ± 0.65

[a] Standard error.

Table 2. Activation Energies (kJ/mol) for Degradation of Chlorophyll and Pheophytin, Determined by Chemical Analysis and Objective Color Measurements

compound	chemical analysis	color analysis (−*a* value)
chlorophyll *a*	71.04 ± 4.89[a]	
chlorophyll *b*	67.11 ± 6.82	
total chlorophyll	69.04 ± 4.78	69.04 ± 3.25[a]
total pheophytin		105.49 ± 4.74

[a] Standard error.

of the obtained degradation data, a fractional conversion technique was used, since a physical property was measured instead of a concentration. Since the $-a$ value reflects the greenness of a sample, this color value was selected as the physical parameter to study the green color loss of broccoli juice. The change in the $-a$ color value could not adequately be described by a first-order fractional conversion model (eq 5). Especially at 90 and 100 °C, a considerable model misspecification was noticed. The estimated a_∞ was furthermore highly dependent on the degradation temperature under consideration and ranged from about −0.3 to +9.0. The latter finding is clearly different from the observations made by Steet and Tong (1996). These authors noted that, when heating pea puree, the $-a$ value asymptotically approached a value of −6.0 ± 0.2, independent of the prevailing degradation temperature. The same value was obtained when the pea puree was acidified, indicating that the a_∞ value corresponded to complete pheophytinization of chlorophyll.

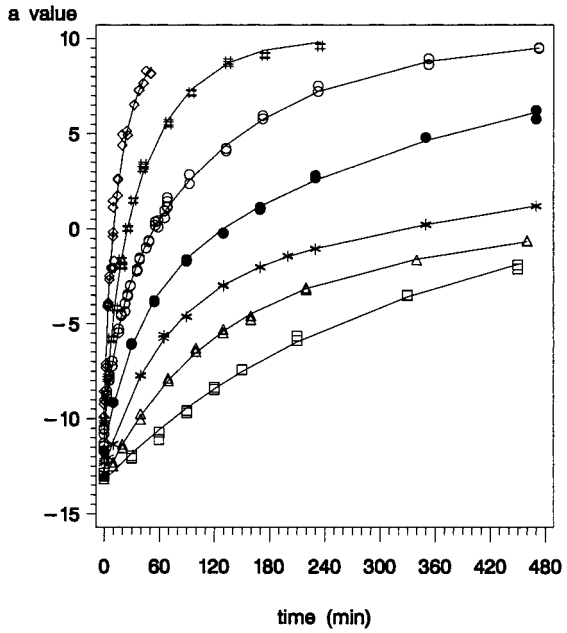

Figure 4. Degradation of the green color in broccoli juice, modeled using a two-step fractional conversion model, at (□) 60, (Δ) 70, (*) 80, (●) 90, (○) 100, (#) 110, and (◇) 120 °C.

Acidifying broccoli juice with oxalic acid yielded an a_∞ value of −2.4, which is definitely lower than the a_∞ values estimated using the first-order fractional conversion model (−0.3 to +9.0) and thus clearly demonstrates that secondary reactions take place when broccoli juice is heated. Since it has been noted by Schwartz and von Elbe (1983) that pheophytin is only an intermediate in the thermal degradation of chlorophyll to pyropheophytin (decarboxymethoxylated magnesium-free chlorophyll derivative), an attempt was made to determine whether the green color degradation could accurately be modeled by means of a consecutive step model. The conventional two-step model was hereto adapted, taking into account the concept of fractional conversion (eq 7). In this

$$P = \left((P_1 - P_\infty) - (P_2 - P_\infty)\left(\frac{k_1}{k_1 - k_2}\right)\right)e^{-k_1 t} + \left((P_2 - P_\infty)\left(\frac{k_1}{k_1 - k_2}\right)\right)e^{-k_2 t} + P_\infty \quad (7)$$

equation, the physical parameter P_∞ symbolizes the $-a$ value when the two-step degradation reaction is completed. Parameter P_2 reflects the physical parameter, that is, the $-a$ value, of the intermediate formed upon heating. Since the $-a$ value of the intermediate (pheophytin) was found to be −2.4 (derived from oxalic acid induced pheophytinization), parameter P_2 was set on −2.4. Initial parameter estimations (P_1, k_1, k_2, P_∞) showed that, for the five highest temperatures (80−120 °C), P_∞ was estimated as 10.30, 10.20, 10.09, 9.68, and 9.50, respectively. Therefore, a numerical value of 10 for P_∞ was used in subsequent estimations. Figure 4 represents the green color degradation, as modeled using eq 7. The estimated rate constants (k_1 and k_2) are summarized in Table 3. For both degradation steps, the temperature dependency of the rate constants could adequately be described by the Arrhenius equation (Figure 5). Correlation coefficients for the linear regression equaled 0.989 and 0.992 for the first and second degradation steps, respectively. Table 2 summarizes the

Table 3. Rate Constants ($\times 10^3$; min^{-1}) for Thermal Color Degradation of Broccoli Juice, Determined by Objective Color Measurements

	physical parameter			
		−*a* value		
	L value	first step	second step	*b* value
60		4.56 ± 0.17[a]		1.86 ± 0.10[a]
70	0.24 ± 0.02[a]	9.08 ± 0.14	0.47 ± 0.02[a]	5.03 ± 0.33
80	0.38 ± 0.03	15.75 ± 0.23	0.85 ± 0.02	17.43 ± 1.80
90	0.94 ± 0.03	26.30 ± 0.72	2.69 ± 0.05	35.28 ± 5.71
100	2.34 ± 0.05	55.42 ± 1.51	6.96 ± 0.17	49.02 ± 5.73
110	5.79 ± 0.12	98.21 ± 4.88	18.27 ± 0.76	60.95 ± 5.64
120	13.95 ± 0.49	231.79 ± 20.91	45.38 ± 3.01	116.51 ± 7.66

[a] Standard error.

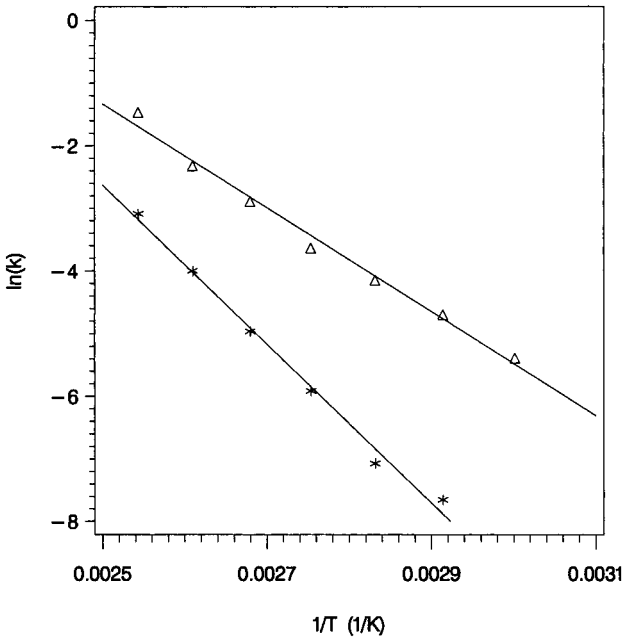

Figure 5. Variation with temperature of the rate constants for the (Δ) first and (*) second degradation steps in green color loss of broccoli juice.

estimated activation energies. The activation energy for the first degradation step, that is, pheophytinization of chlorophyll, was very similar to that obtained for chlorophyll degradation using the procedure of Vernon (1960). Using the latter chemical assay, the subsequent pheophytin to pyropheophytin conversion was most probably not observed because of their similar light absorption in the visible spectrum (Pennington et al., 1964). Consequently, the concentrations represented in Figure 1 refer in fact to both pheophytin and pyropheophytin. The chemical analysis thus reveals information on the degradation of chlorophyll to pheophytin only. The similarity between the activation energies for chlorophyll to pheophytin conversion, derived by chemical analysis and objective color measurements, supports the two-step degradation mechanism set forth by Schwartz and von Elbe (1983).

It was investigated whether the first step in the greenness degradation of broccoli juice was due to total chlorophyll degradation or to pheophytinization of chlorophyll *a* in particular. According to Steet and Tong (1996), heat-induced loss of green color is a consequence of both chlorophyll *a* and *b* degradation. Sweeney and Martin (1958) suggested, on the other hand, that the loss of green color is mainly due to pheophytinization of chlorophyll *a*, because chlorophyll *a* has an intense

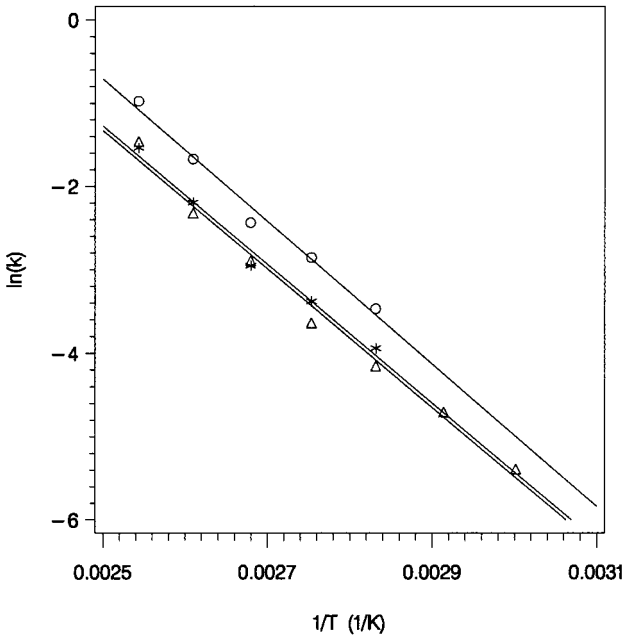

Figure 6. Variation with temperature of the rate constants for (Δ) the first degradation step in green color loss and the "normalized" degradation rate constants for (*) total chlorophyll and (○) chlorophyll *a*.

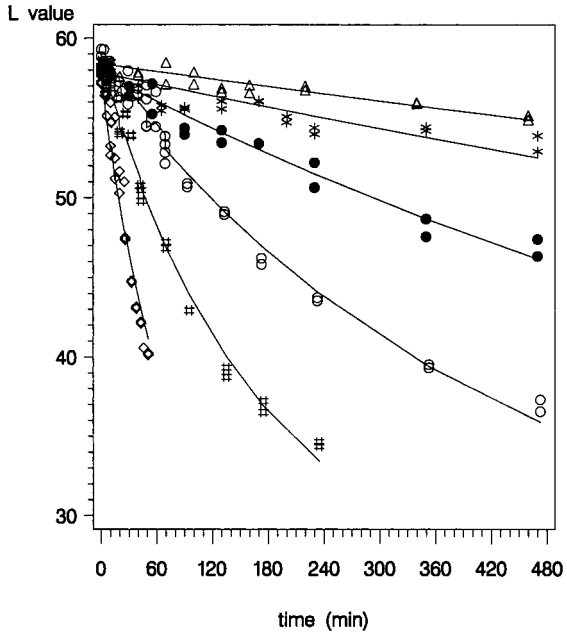

Figure 7. Change in color value *L* of broccoli juice upon heating, modeled using a first-order fractional conversion model, at (Δ) 70, (*) 80, (●) 90, (○) 100, (#) 110, and (◇) 120 °C.

blue-green color whereas chlorophyll *b* is characterized by a yellow-green color, as is pheophytin. The degradation curves of chlorophyll degradation in broccoli juice were hereto normalized. Initial total chlorophyll and chlorophyll *a* content equaled on average 4.65 and 3.45 μg/mL, respectively. The $-a$ value of broccoli juice varied, on the other hand, from −13.0 to −2.4 upon pheophytinization (derived from oxalic acid induced pheophytinization). To compare the degradation rate constants determined by means of chemical analysis and objective color measurements, the *k* values for pheophytinization determined using the chemical analysis were thus multiplied by 2.28 or 3.07 in the case of total chlorophyll and chlorophyll *a* content, respectively. On the basis of the similarity of the normalized *k* values for pheophytinization of the total chlorophyll content, measured by means of the chemical assay, and the first-step degradation rate constants obtained by objective color measurements (Figure 6), it was concluded that the loss of greenness in broccoli juice upon heating is due to the degradation of both chlorophyll *a* and *b*.

In addition to the variation of the $-a$ value as a function of heating time, attention was paid to the variation of the *L* and *b* color values. Although addition of oxalic acid, inducing pheophytinization, did not affect the *L* and *b* color values, changes in both color values were observed when broccoli juice was heated. The changes in *L* and *b* color values may therefore be due to pheophytin−pyropheophytin conversion or to degradation/reaction of other compounds present in the broccoli juice. The changes in the latter color values could accurately be modeled using the first-order fractional model (eq 5). The P_∞ value was estimated as 25 for the *L* value and 27.5 for the *b* value. Since the initial *L* and *b* values equaled on average 58 and 32, it is clear that, contrary to the *a* and *L* color values, the change in *b* value was only very limited. This finding is in agreement with the recent observations by Ihl et al. (1998) for artichokes. Figure 7 illustrates the decrease in *L* value, modeled using the first-order fractional conversion model. The corresponding degradation rate constants are given in Table 3, together with the degradation rate constants when the *b* value is used as physical property. In both cases, the temperature dependence of the estimated degradation rate constants could satisfactorily be described by the Arrhenius equation (Figure 8). The correlation coefficients for the linear regression analysis were 0.985 and 0.952 for the change in *L* and *b* values, respectively. The estimated activation energies equaled 93.84 ± 5.75 and 72.50 ± 7.25 kJ/mol, respectively.

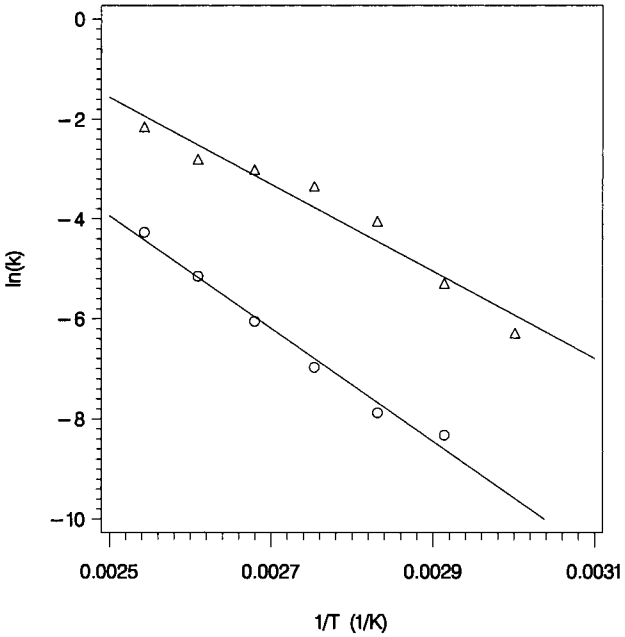

Figure 8. Variation with temperature of the degradation rate constants when the (○) *L* and (Δ) *b* color values are monitored.

CONCLUSION

Upon thermal treatment chlorophyll degrades to pheophytin, which in turn decomposes to other degra-

dation products. Both chlorophyll and pheophytin conversions appear to be first-order degradation processes. The conversion of chlorophylls *a* and *b* upon heating results in a marked decrease of the $-a$ color value. Heating of the broccoli juice furthermore brought about a minor decrease of the *b* color value and a major decrease of the *L* color value.

The present study clearly shows the advantage of objective color measurements for studying the degradation of chlorophyll, as compared to conventional chemical analyses. From the point of view of consumers, the (green) color per se is more important than the residual chlorophyll content.

ABBREVIATIONS USED

a, tristimulus color value; *b*, tristimulus color value; *C*, chlorophyll concentration (μg/mL); C_0, initial chlorophyll concentration (μg/mL); E_a, activation energy (kJ/mol); *k*, first-order degradation rate constant (min^{-1}); k_1, first-order degradation rate constant (min^{-1}) for the first degradation step; k_2, first-order degradation rate constant (min^{-1}) for the second degradation step; k_{ref}, first-order degradation rate constant at T_{ref} (min^{-1}); *L*, tristimulus color value; *P*, physical property at time *t*; P_0, physical property at $t = 0$; P_1, physical property when chlorophyll is unaffected; P_2, physical property when chlorophyll is converted to the intermediate (pheophytin); P_∞, physical property when the degradation reaction is completed; *R*, universal gas constant (8.314 J K^{-1} mol^{-1}); *t*, heating time (min); *T*, temperature (K); T_{ref}, reference temperature (K); *V*, volume; *X*, tristimulus color value; *Y*, tristimulus color value; *Z*, tristimulus color value; ΔA_x, absorbance difference of the nonacidified and acidified pigment solution at *x* nm.

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