Thermal and Pressure−Temperature Degradation of Chlorophyll in Broccoli (*Brassica oleracea* L. *italica*) Juice: A Kinetic Study

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Heat and combined pressure−temperature degradation of chlorophyll in broccoli juice have been studied on a kinetic basis. A treatment at 100 °C and atmospheric pressure during 37 min resulted in a 90% decrease in total chlorophyll content of broccoli juice. An extreme pressure stability of both chlorophylls *a* and *b* at room temperature was observed. Significant reductions in chlorophyll content were noticed only when pressure was combined with temperatures exceeding 50 °C. Chlorophyll degradation reactions at atmospheric as well as at elevated pressures could be adequately described by assuming a first-order decay. Chlorophyll *a* degradation occurred more rapidly as compared to chlorophyll *b* degradation under all pressure−temperature combinations tested (1−800 MPa/50−120 °C). Degradation rates increased with temperature according to the Arrhenius law at all pressures tested.

Keywords: *Chlorophyll; broccoli (Brassica oleracea L. italica); kinetics; temperature; pressure*

INTRODUCTION

In previous decades, the primary objective of food manufacturers was to produce safe shelf stable products and food quality was considered of secondary importance; however, throughout the 1980s to the present day, the food industry is driven by consumers' desire for high-quality, minimally processed, additive-free, shelf stable, convenient, and safe food products. As a result, existing thermal preservation technologies are improved (e.g., better process control) or adjusted (e.g., aseptic processing, Ohmic and microwave heating), or new physical food preservation methods are introduced. In this context, the use of high hydrostatic pressure (HHP) receives plentiful interest from the food industry because it has been demonstrated experimentally that HHP at room temperature can inactivate vegetative microorganisms and food deteriorative enzymes while only slightly affecting nutritional and sensorial food quality aspects (e.g., vitamin content, flavor, and color) (Farr, 1990; Cheftel, 1991; Hoover, 1993; Knorr, 1993; Hayakawa et al., 1994; Mozhaev et al., 1994). Due to the extreme pressure stability of the spores of some species, a combination of pressure with other, ideally synergistic, preservation technologies is required if pressure sterilization of low-acid foods is to be pursued. For this purpose, the effect of high pressure and elevated temperature on microbial as well as enzymatic inactivation is under investigation. Experimental systematic and quantitative studies on the effect of HHP and elevated temperature on food quality carriers are, however, rather scarce (Van den Broeck et al., 1998).

Color is regarded as a major food quality attribute in determining product acceptability, the degree of importance being dependent upon the particular food system under consideration (Villota and Hawkes, 1986). One of the disadvantages of thermal treatments is the modification of pigments under the influence of heat, which results in changes in the original color of the food material. With regard to green color, a common change in processed green plants is the degradation of chlorophyll (green color) to pheophytins (gray-brown color), a process that is highly associated with a decreased product quality. Hence, a challenge to food processors is to prevent or at least minimize this green color degradation to produce high-quality vegetable products. Methods that have been studied in the past include among others enzymatic conversion of chlorophylls to chlorophyllides (Clydesdale and Francis, 1968) and pH adjustment combined with high-temperature short-time processing (Buckle and Edwards, 1970).

The objective of the present study was to verify whether high-pressure processing can result in an improved chlorophyll retention as compared to thermal processing. To predict chlorophyll degradation during processing, accurate kinetic parameters are essential. Hence, in the present study the effect of temperature and of high pressure on chlorophyll degradation in a vegetable product, namely *Brassica oleracea* L. *italica* (broccoli), has been investigated on a kinetic basis.

MATERIALS AND METHODS

Sample Preparation. Broccoli was purchased from a local auction. The broccoli heads were squeezed to prepare a batch of 2.5 L of broccoli juice. The broccoli juice was quickly frozen in 30 mL tubes using liquid nitrogen and kept under liquid nitrogen until use. Frozen broccoli juice was thawed overnight at 7 °C, protected from light.

Thermal Treatment. Heat degradation kinetics of chlorophyll was determined by monitoring the chlorophyll concentration as a function of inactivation time at constant

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temperature (steady-state procedure). To avoid heating and cooling lags, broccoli juice was contained in small glass vials of 0.8 mL (Chrompack) and heated during preset time intervals in an oil bath at a constant inactivation temperature (ranging from 80 to 120 °C). Immediately upon withdrawal from the oil bath, samples were cooled in ice water until determination of the remaining chlorophyll concentration as described below.

Combined High Pressure−Temperature Treatment. The kinetic parameter values for the high-pressure−temperature degradation of chlorophyll were determined by monitoring the chlorophyll concentration as a function of treatment time at constant pressure and temperature. Isobaric−isothermal experiments have been performed in a multivessel high-pressure apparatus (Resato, Roden, The Netherlands) that allows pressurization up to 1000 MPa in combination with temperatures from −35 to 100 °C. The pressure transmitting fluid is a mixture of oil and glycol (TR15, Resato). As the high-pressure equipment contains eight thermostated (Cryostat Haake) vessels of 8 mL, each vessel being provided with a type J thermocouple to measure temperature evolution during processing inside the vessel, eight samples can be treated simultaneously at a desired pressure and temperature during different time periods.

In the present study, kinetic experiments were performed in the pressure range from 200 to 800 MPa combined with temperatures in the range from 30 to 80 °C. Broccoli juice (2.5 mL) was contained in flexible bags and placed in the preheated pressure vessels. The pressure was always built up at a constant rate (±90 MPa/min) until the preset pressure was reached, whereupon the valves of the individual vessels were closed and the central tubing was decompressed. The valve of the first vessel was opened ∼5 min [time to reach equilibrium conditions in terms of temperature and pressure (Weemaes et al., 1997)] after the achievement of the preset pressure. As during the pressure buildup, which is accompanied by a temperature increase due to adiabatic heating of the pressure medium, and during the equilibrium time of 5 min, chlorophyll in the broccoli juice can already be partially degraded, the residual chlorophyll concentration of the sample in this vessel was taken as initial response value (X_0), that is, the chlorophyll concentration at $t = 0$. From this moment on, the treatment can be considered as an isobaric−isothermal treatment. Afterward, the vessels were decompressed at preset time intervals. After the pressure−temperature treatment, broccoli samples were immediately transferred to ice water until determination of the residual chlorophyll concentration as described below.

Determination of Chlorophyll Concentration. After the degradation treatment, chlorophyll *a* concentration, chlorophyll *b* concentration, and the total chlorophyll content were determined as described by Vernon (1960). Chlorophyll was extracted from the broccoli juice with acetone (80% v/v) in a darkened room. After 15 min, the solution was filtered (Schleicher & Schuell 595) and consecutively centrifuged at 12100*g* for 10 min. To half of the pigment solution was added saturated oxalic acid [2% (v/v)] in 80% (v/v) acetone to fully convert chlorophyll to pheophytin. The acidified as well as the nonacidified pigment solutions were kept at room temperature in a darkened room for exactly 3 h. Afterward, the absorbances of the acidified and the nonacidified solutions were determined at both 662 and 645 nm (Ultrospec K, Pharmacia LKB Biochrom Ltd.). The concentrations of chlorophylls *a* and *b* were calculated using the conversion formulas of Vernon (1960) (eqs 1 and 2), which take into account the possible presence of pheophytins *a* and *b* in the extracted system. The total chlorophyll concentration was calculated by summation of the chlorophyll *a* and chlorophyll *b* concentrations.

$$[\text{Chl } a] = 25.38(\Delta A662) + 3.64(\Delta A645) \quad (1)$$

$$[\text{Chl } b] = 30.38(\Delta A645) - 6.58(\Delta A662) \quad (2)$$

$\Delta A662$ is the absorbance difference of a nonacidified solution and an acidified one at 662 nm, and $\Delta A645$ is the absorbance difference of a nonacidified solution and an acidified one at 645 nm.

Data Analysis. Results of thermal and combined pressure−temperature treatments were processed into kinetic parameter values, that is, reaction order (n), rate constants (k), activation energies (E_a), and activation volumes (V_a).

In general, an nth-order reaction rate equation can be written as

$$dX/dt = -kX^n \quad (3)$$

where X is the response property, k the rate constant, t the treatment time, and n the reaction order.

Chlorophyll degradation during thermal processing has been frequently described in the literature as a first-order reaction (Schwartz and Von Elbe, 1983; Canjura et al., 1991; Schwartz and Lorenzo, 1991). For a first-order reaction, the integrated form of eq 3 for a decay process at constant pressure and temperature (i.e., rate constant independent of time) is given by

$$X/X_0 = \exp(-kt) \quad (4)$$

where X_0 is the response value at $t = 0$ (i.e., the concentration of chlorophyll at $t = 0$) and X the residual response value after treatment (i.e., the residual concentration of chlorophyll). For each pressure−temperature combination, the rate constant (k) of the chlorophyll degradation reaction was estimated on the basis of eq 4 using a nonlinear regression approach (*SAS/STAT User's Guide*, 1994).

The temperature dependence of the chlorophyll degradation rate constants (k) at atmospheric pressure has been adequately described in the literature using an activation energy E_a, as given in the Arrhenius relationship (eq 5) (Schwartz and Von Elbe, 1983; Canjura et al., 1991; Schwartz and Lorenzo, 1991; Steet and Tong, 1996).

$$k = k_{ref} \exp\left[\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right] \quad (5)$$

k_{ref} is the rate constant at reference temperature T_{ref}, E_a is the activation energy, and R is the universal gas constant (R = 8.314 J/K mol). The activation energy at a certain pressure was estimated on the basis of eq 5 using a nonlinear regression analysis (*SAS/STAT User's Guide*, 1994).

The pressure dependence of the rate constant (k) at a certain temperature is commonly described as an activation volume V_a, as given in the Eyring relation

$$k = k_{ref} \exp\left[-\frac{V_a}{RT}(P - P_{ref})\right] \quad (6)$$

where k_{ref} is the rate constant at reference pressure P_{ref}, V_a is the activation volume at a certain temperature, T is the absolute temperature, and R is the universal gas constant (R = 8.314 cm^3·MPa/K mol).

RESULTS AND DISCUSSION

In all higher plants chlorophylls *a* and *b* are found to be, respectively, the principal and the minor chlorophyll, occurring in an approximate ratio of 3:1. For the broccoli juice used in this study, the average ratio of chlorophyll *a* versus chlorophyll *b* was 2.9:1. The total chlorophyll concentration of the broccoli juice was 9.16 μg/mL (6.81 μg/mL chlorophyll *a* and 2.35 μg/mL chlorophyll *b*).

Thermal Degradation Kinetics of Chlorophyll. The thermal degradation of chlorophylls *a* and *b* in broccoli juice was investigated at atmospheric pressure in a temperature range between 80 and 120 °C. Equation 4 was linearized using a logarithmic data transformation to be able to easily interpret graphically and statistically the validity of a first-order inactivation model. From a loglinear plot of the relative residual

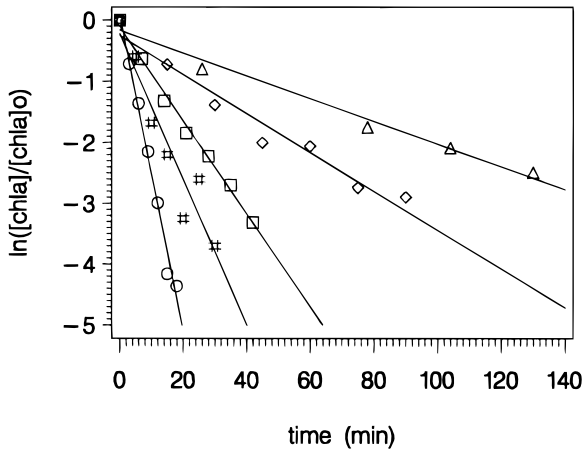

Figure 1. Thermal degradation of chlorophyll *a* at atmospheric pressure and 80 °C (△), 90 °C (◇), 100 °C (□), 110 °C (#), and 120 °C (○).

response value [ln(X/X_0)] as a function of inactivation time at constant temperature and pressure, it was verified whether thermal degradation of chlorophyll could be adequately described by a first-order reaction. On the basis of the coefficients of determination R^2 ($R^2 > 0.96$ for chlorophyll *a* and $R^2 > 0.93$ for chlorophyll *b*), the visual absence of trends in the residu-plots (Straume and Johnson, 1992), and the runs test (Mannervik, 1982) for randomness of residu-plots, thermal degradation of chlorophyll could be adequately described by a first-order reaction. As an example, a loglinear plot of relative chlorophyll *a* retention versus degradation time is depicted in Figure 1. Kinetics of thermal chlorophyll degradation have been presented extensively in the literature. These studies revealed that the rate of this degradation followed a first-order kinetic model (Schwartz and Von Elbe, 1983; Canjura et al., 1991; Schwartz and Lorenzo, 1991; Steet and Tong, 1996).

The logarithmic transformation of the experimental data was not applied for kinetic parameter estimation as this nonlinear data transformation might alter the Gaussian distribution of experimental uncertainties—an assumption inherent to least-squares fitting. As a consequence, rate constants of the chlorophyll degradation process at all temperatures tested were estimated on the basis of eq 4 using nonlinear regression analysis and are presented in Table 1. The model fitting of thermal degradation of chlorophyll *b* at atmospheric pressure is depicted in Figure 2.

With regard to the ranking in heat stability of chlorophylls *a* and *b*, it can be concluded that chlorophyll *a* is less thermostable (as indicated by a higher rate constant at constant temperature for chlorophyll *a* degradation) as compared to chlorophyll *b*, and hence chlorophyll *a*, which is the major chlorophyll compound in green plants, degrades more quickly. Chlorophyll *a* has previously been described in the literature as being less thermostable than chlorophyll *b* in processed broccoli (Sweeney and Martin, 1958), pea puree (Buckle and Edwards, 1970), spinach (Schwartz and Von Elbe, 1983; Canjura et al., 1991; Schwartz and Lorenzo, 1991), and peas (Steet and Tong, 1996). Schwartz and Von Elbe (1983) found rate constants at 121 °C of 0.178 and 0.085 min^{-1}, respectively, for chlorophyll *a* and *b* degradation in spinach puree, whereas Canjura et al. (1991) obtained rate constants at 100 °C of 0.132 and 0.024 min^{-1}, respectively, for chlorophyll *a* and *b* degradation in

Table 1. Rate Constants for Degradation of Chlorophyll *a*, Chlorophyll *b*, and Total Chlorophyll Content in Broccoli Juice due to a Thermal or a Combined Pressure–Temperature Treatment

P (MPa)	*T* (°C)	Chl *a* *k* (10^{-2} min^{-1})	Chl *b* *k* (10^{-2} min^{-1})	total Chl *k* (10^{-2} min^{-1})
0.1	80	2.51 ± 0.26[a]	0.57 ± 0.03[a]	1.59 ± 0.19[a]
	90	4.41 ± 0.24	1.20 ± 0.05	2.95 ± 0.20
	100	8.84 ± 0.24	2.46 ± 0.11	6.15 ± 0.31
	110	14.13 ± 0.95	4.73 ± 0.27	10.29 ± 0.65
	120	23.60 ± 0.35	9.07 ± 0.78	17.66 ± 0.36
200	60	0.45 ± 0.02	nd[b]	0.33 ± 0.02
	70	1.60 ± 0.15	0.57 ± 0.01	1.13 ± 0.09
	80	4.11 ± 0.43	1.59 ± 0.05	2.76 ± 0.19
500	60	0.52 ± 0.02	0.10 ± 0.01	0.38 ± 0.02
	70	1.48 ± 0.09	0.56 ± 0.03	1.13 ± 0.04
	80	4.04 ± 0.24	2.91 ± 0.07	3.45 ± 0.13
700	60	0.64 ± 0.03	0.23 ± 0.01	0.50 ± 0.02
	70	1.67 ± 0.06	0.84 ± 0.06	1.37 ± 0.05
	80	3.99 ± 0.41	2.78 ± 0.16	3.36 ± 0.23
800	50	0.20 ± 0.02	nd	0.16 ± 0.02
	60	0.57 ± 0.04	0.19 ± 0.02	0.44 ± 0.03
	70	1.91 ± 0.05	0.96 ± 0.04	1.45 ± 0.04
	80	3.48 ± 0.02	3.67 ± 0.14	3.94 ± 0.27

[a] Asymptotic standard error of regression. [b] nd, not determined.

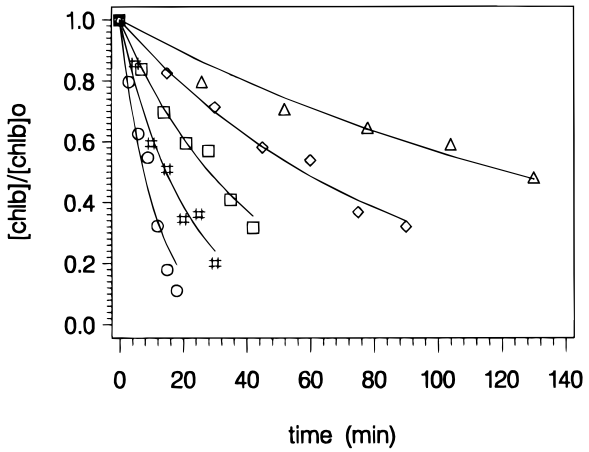

Figure 2. Thermal degradation of chlorophyll *b* at atmospheric pressure and 80 °C (△), 90 °C (◇), 100 °C (□), 110 °C (#), and 120 °C (○).

spinach puree. Steet and Tong (1996) studied the kinetics of chlorophyll degradation in pureed green peas and obtained rate constants at 80 °C of 0.0173 and 0.0083 min^{-1} for chlorophylls *a* and *b*, respectively. If these literature data are compared with the rate constants obtained in the present study (Table 1), it can be concluded that, although it is known that rate constants are influenced by the environmental conditions of the aspect studied, the rate constants for thermal chlorophyll degradation in different vegetables are of the same order of magnitude.

The temperature dependence of the chlorophyll degradation rate constants at atmospheric pressure could be accurately described by an Arrhenius relationship, for chlorophyll *a*, chlorophyll *b*, and total chlorophyll content (Figure 3), yielding activation energies of 67.49 ± 1.75 kJ/mol for total chlorophyll content and 63.28 ± 1.80 kJ/mol for chlorophyll *a* degradation and a higher activation energy of 80.14 ± 0.50 kJ/mol for chlorophyll *b* degradation (Table 2). This ranking in activation energy for chlorophyll *a* and *b* degradation is, however, not always reported in the literature. Schwartz and Von

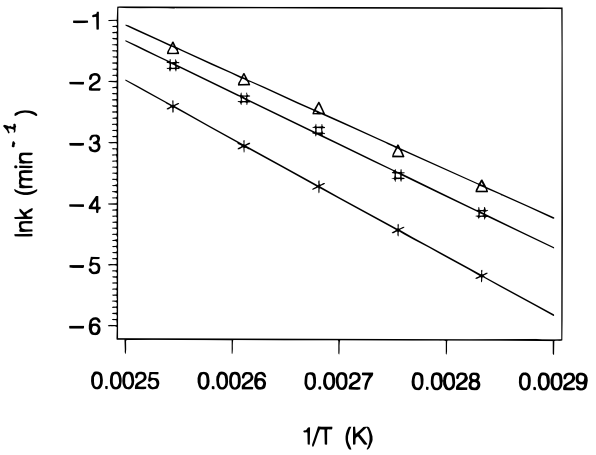

Figure 3. Arrhenius plot for thermal degradation of chlorophyll *a* (△), chlorophyll *b* (*), and total chlorophyll content (#) in the temperature range from 80 to 120 °C.

Table 2. Activation Energies for Degradation of Chlorophyll *a*, Chlorophyll *b*, and Total Chlorophyll Content in Broccoli Juice at Different Pressures (0.1−800 MPa)

P (MPa)	Chl *a* E_a (kJ/mol)	Chl *b* E_a (kJ/mol)	total Chl E_a (kJ/mol)
0.1	63.28 ± 1.80[a]	80.14 ± 0.50[a]	67.49 ± 1.75
200	99.32 ± 6.24	nd[b]	94.87 ± 6.70
500	100.72 ± 0.13	165.84 ± 0.03	111.14 ± 2.53
700	88.30 ± 1.02	120.63 ± 0.70	91.72 ± 1.43
800	75.88 ± 10.54	136.65 ± 3.37	102.65 ± 1.90

[a] Asymptotic standard error of regression. [b] nd, not determined.

Elbe (1983), who investigated the degradation of chlorophyll in heat-processed spinach in the temperature range from 116 to 126 °C, obtained activation energies of 105.3 kJ/mol for chlorophyll *a* and 94.1 kJ/mol for chlorophyll *b*. Thermal degradation of chlorophyll in spinach puree has been studied in the temperature range from 100 to 145 °C by Canjura et al. (1991), yielding activation energies of 62.7 kJ/mol for chlorophyll *a* and 92.0 kJ/mol for chlorophyll *b*. Steet and Tong (1996) determined the kinetics of heat-induced chlorophyll degradation in pureed green peas in the temperature domain from 70 to 90 °C and obtained activation energies of 81.5 kJ/mol for chlorophyll *a* and 71.5 kJ/mol for chlorophyll *b* degradation.

Pressure−Temperature Degradation Kinetics of Chlorophyll. Kinetics of isobaric−isothermal degradation of chlorophyll was studied in the pressure range from 200 to 800 MPa at temperatures ranging from 30 to 80 °C.

An extreme stability of chlorophyll toward pressure processing was observed. Even after treatment times of 4 h at 800 MPa and 40 °C, no reduction in chlorophyll *a* or chlorophyll *b* content could be noticed. Only for high-pressure treatments combined with temperatures exceeding 50 °C has a significant reduction in chlorophyll concentration been observed. Hence, high-pressure treatments at room temperature or slightly above are to be recommended to minimize chlorophyll degradation during processing and consequently minimize green color losses of processed vegetable products. This observation can be attributed to the stability of the covalent structure of chlorophylls to high pressure due to the negligible compressibility of covalent bonds (Hayashi, 1989; Knorr, 1993; Mozhaev et al., 1994).

Because of the pressure resistance of spores and some food deteriorative enzymes at room temperature, a combination of high pressure with other treatments (e.g., heat treatment) is needed to obtain sterile and shelf stable food products. Hence, it is worthwhile to investigate the pressure stability of chlorophyll at elevated temperatures in detail.

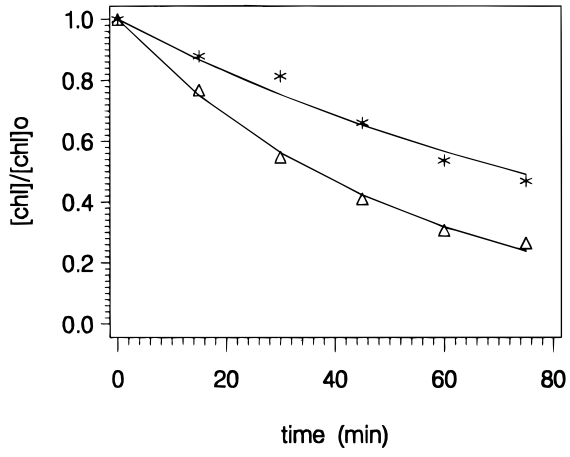

Figure 4. Pressure−temperature degradation of chlorophyll *a* (△) and chlorophyll *b* (*) at 800 MPa and 70 °C. X_0 and X are total and residual chlorophyll concentrations, respectively.

From semilogarithmic plots of chlorophyll retention in broccoli juice as a function of treatment time at constant pressure and temperature, it was ensured that the pressure−temperature-induced chlorophyll degradation process followed a first-order decay. Rate constants have been estimated by nonlinear regression analysis using eq 4 (Table 1). It can again be noticed that the rate constants for chlorophyll *a* degradation are higher than the ones for chlorophyll *b* degradation under all conditions tested. Hence, pressure−temperature degradation of chlorophyll *b* occurs more slowly than chlorophyll *a* degradation, as can be seen from Figure 4.

The relative difference in rate constants for chlorophyll *a* and chlorophyll *b* degradation at a certain pressure−temperature combination decreases as temperature increases (Table 1), indicating a different activation energy for chlorophyll *a* degradation as compared to chlorophyll *b* degradation.

The reproducibility of the determination of chlorophyll degradation rate constants has been tested by performing independent duplicate isobaric−isothermal experiments at different pressure−temperature combinations. On average, rate constants varied 5.6% between duplicate experiments.

From the linearity of the plots of the logarithm of the rate constants at constant pressure versus the reciprocal of the absolute temperature, it was concluded that the Arrhenius relationship was valid at all pressures tested. Activation energies for degradation of chlorophyll *a*, chlorophyll *b*, and total chlorophyll content are estimated on the basis of eq 5 by nonlinear regression analysis and are presented in Table 2. No clear tendency in the variation of the activation energy for chlorophyll degradation with pressure was observed. As in the case of thermal treatment, it can be observed that for all pressures tested the activation energy for chlorophyll *a* degradation is lower as compared to that for chlorophyll *b* degradation, indicating that the rate of chlorophyll *b* degradation is more temperature sensitive. For chlorophyll *b* degradation, the activation energy at

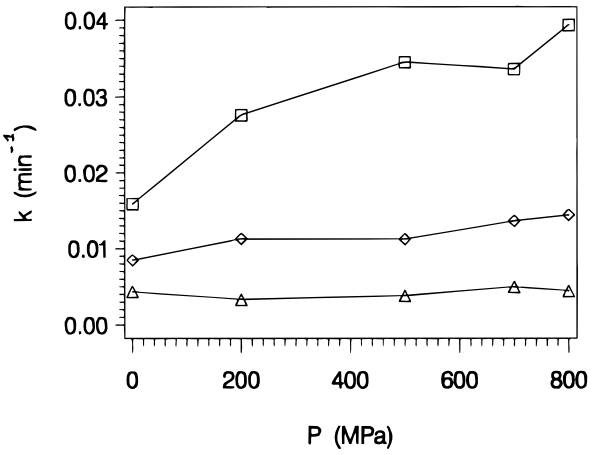

Figure 5. Rate constants of pressure–temperature degradation of total chlorophyll content at 60 °C (△), 70 °C (◇), and 80 °C (□) as a function of pressure.

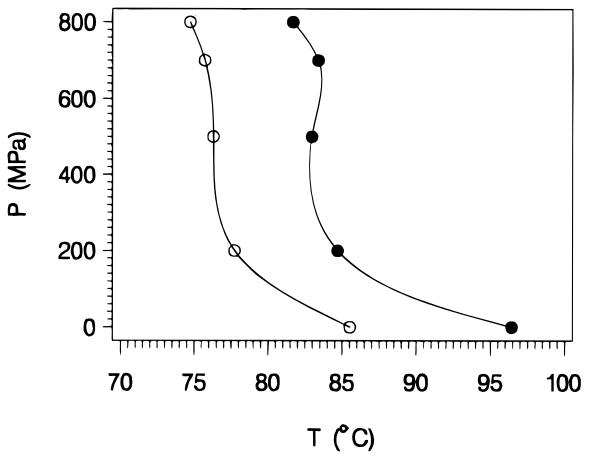

Figure 6. Pressure–temperature combinations that will result in a 90% decrease in total chlorophyll content after process times of 50 min (●) and 100 min (○) at the selected pressure–temperature combination.

elevated pressure is significantly higher than the one at atmospheric pressure. Hence, rate constants for chlorophyll *b* degradation are less temperature sensitive at atmospheric pressure.

The pressure dependence of the rate constants at constant temperature could not be described accurately by the Eyring relationship (eq 6): a systematic deviation from a linear behavior of the natural logarithm of the rate constants versus pressure has been observed.

When the rate constants for chlorophyll degradation at a certain temperature are plotted as a function of pressure (Figure 5), a synergistic effect of pressure and temperature on chlorophyll degradation can be noticed. At 70 °C, increasing the pressure from 200 to 800 MPa results in an increase in rate constants of 19.4% for chlorophyll *a* and 68.4% for chlorophyll *b*. At 80 °C, a significant increase in rate constants due to pressurization is observed up to 500 MPa. A further increase in pressure from 500 to 800 MPa does not accelerate the chlorophyll degradation reaction to a large extent. From Figure 5, it is observed that a pressure increase from 0.1 to 500 MPa has a more pronounced effect on the chlorophyll degradation rate constant as the inactivation temperature increases. Whereas a temperature increase of 10 °C (e.g., from 70 to 80 °C) greatly increases the chlorophyll degradation rate constant, a pressure increase of 100 MPa has only a minor influence on the degradation rates.

For combined pressure–temperature treatments, only one limited qualitative study of chlorophyll degradation has been found in the literature. According to Salanski et al. (1997), the change in absorbance of pigments in water solutions, including chlorophyll, exposed to pressures up to 800 MPa at ambient temperature for 30 min did not exceed 15%, which agrees with our results. Quantitative studies with regard to the effect of pressure processing on chlorophyll concentration or on green color retention of food products are completely lacking.

On the basis of the experimentally determined kinetic data on chlorophyll degradation, equivalent pressure–temperature combinations with respect to chlorophyll retention can be calculated theoretically. For example, the rate of chlorophyll degradation when processing at 90 °C and atmospheric pressure equals the chlorophyll degradation rate of pressure processing at 700 MPa and 78.4 °C or alternatively at 800 MPa and 77.2 °C. In Figure 6, combined pressure–temperature treatments are depicted that will result in a 90% decrease in chlorophyll concentration after processing times of 50 and 100 min at the specified pressure–temperature combination. From Figure 6, it is again apparent that temperature is the major rate-determining extrinsic process factor; pressure has only a minor influence on the chlorophyll degradation rate. Increasing the pressure above 500 MPa has only a slight influence on the rate of chlorophyll degradation in broccoli juice.

In conclusion, as the chlorophyll concentration in broccoli juice is not decreased by the application of pressures up to 800 MPa combined with temperatures up to 50 °C, high-pressure processing below 50 °C can offer a major advantage as a preservation method in comparison with classical pasteurization processes to obtain vegetable products that keep their original fresh green color. The experimentally determined kinetic data on pressure–temperature-induced chlorophyll degradation form the basis to optimize thermal and combined pressure–temperature treatments of broccoli in terms of maximal chlorophyll retention.

LITERATURE CITED

Buckle, K. A.; Edwards, R. A. Chlorophyll, Colour and pH Changes in H.T.S.T. Processed Green Pea Puree. *J. Food Technol.* **1970**, *5*, 173–186.

Canjura, F. L.; Schwartz, S. J.; Nunes, R. V. Degradation Kinetics of Chlorophylls and Chlorophyllides. *J. Food Sci.* **1991**, *56*, 1639–1643.

Cheftel, J. Applications Des Hautes Pressions en Technologie Alimentaire (Applications of High Pressure in Food Technology). *Ind. Aliment. Agric.* **1991**, *108* (3), 141–153.

Clydesdale, F. M.; Francis, F. J. Chlorophyll Changes in Thermally Processed Spinach as Influenced by Enzyme Conversion and pH Adjustment. *Food Technol.* **1968**, 793.

Farr, D. High-Pressure Technology in the Food Industry. *Trends Food Sci. Technol.* **1990**, *1*, 14–16.

Hayakawa, I.; Kanno, T.; Tomita, M.; Fujio, Y. Application of High Pressure for Spore Inactivation and Protein Denaturation. *J. Food Sci.* **1994**, *59*, 159–163.

Hayashi, R. Application of High Pressure to Food Processing and Preservation: Philosophy and Development. In *Engineering and Food;* Spiess, W., Schubert, H., Eds.; Elsevier Applied Science: London, England, 1989; pp 815–826.

Hoover, D. G. Pressure Effects on Biological Systems. *Food Technol.* **1993**, 150–155.

Knorr, D. Effects of High-Hydrostatic-Pressure Processes on Food Safety and Quality. *Food Technol.* **1993**, 156–163.

Mannervik, B. Regression analysis, experimental error, and statistical criteria in the design and analysis of experiments for discrimination between rival kinetic models. *Methods Enzymol.* **1982**, *87*, 370−390.

Mozhaev, V. V.; Heremans, K.; Frank, J.; Masson, P.; Balny, C. Exploiting the Effects of High Hydrostatic Pressure in Biotechnological Applications. *Trends Biotechnol.* **1994**, *12*, 493−501.

Salanski, P.; Jurczak, J.; Fonberg-Broczek, M.; Szczepek, J. Influence of Ultra-high Pressure on Chosen Natural Plant Pigments. In *High-Pressure Research in the Biosciences and Biotechnology;* Heremans, K., Balny, C., Frank, J., Hayashi, R., Hendrickx, M., Ludwig, H., Eds.; Leuven University Press: Leuven, Belgium, 1997; pp 343−345.

SAS/STAT User's Guide, version 6; SAS Institute Inc.: Cary, NC, 1994; pp 1135−1194.

Schwartz, S. J.; Lorenzo, T. V. Chlorophyll Stability During Continuous Aseptic Processing and Storage. *J. Food Sci.* **1991**, *56*, 1059−1062.

Schwartz, S. J.; Von Elbe, J. H. Kinetics of Chlorophyll Degradation to Pyropheophytin in Vegetables. *J. Food Sci.* **1983**, *48*, 1303−1306.

Steet, J. A.; Tong, C. H. Degradation Kinetics of Green Color and Chlorophylls in Peas By Colorimetry and HPLC. *J. Food Sci.* **1996**, *61*, 924−931.

Straume, M.; Johnson, M. L. Analysis of residuals: criteria for determining Goodness-of-fit. *Methods Enzymol.* **1992**, *210*, 87−105.

Sweeney, J. P.; Martin, M. Determination of Chlorophyll and Pheophytin in Broccoli Heated by Various Procedures. *Food Res.* **1958**, *23*, 635−647.

Van den Broeck, I.; Ludikhuyze, L.; Weemaes, C.; Van Loey, A.; Hendrickx, M. Kinetics for isobaric−isothermal degradation of L-ascorbic acid. *J. Agric. Food Chem.* **1998**, *46*, 2001−2006.

Vernon, L. P. Spectrophotometic Determination of Chlorophylls and Pheophytins in Plant Extracts. *Anal. Chem.* **1960**, *32*, 1144−1150.

Villota, R.; Hawkes, J. Kinetics of Nutrients and Organoleptic Changes in Foods during Processing. In *Physical and chemical Properties of Foods*; Okos, M. R., Ed.; ASAE: St. Joseph, MI, 1986; pp 266−366.

Weemaes, C. A.; De Cordt, S. V.; Ludikhuyze, L. R.; Van den Broeck, I.; Hendrickx, M. E.; Tobback, P. P. Influence of pH, benzoic acid, EDTA and glutathione on the pressure and/or temperature inactivation kinetics of mushroom polyphenoloxidase. *Biotechnol. Prog.* **1997**, *13*, 25−32.

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