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Model Studies on the Stability of Folic Acid and 5-Methyltetrahydrofolic Acid Degradation during Thermal Treatment in Combination with High Hydrostatic Pressure

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Stability of folic acid and 5-methyltetrahydrofolic acid in phosphate buffer (0.2 M; pH 7) toward thermal (above 65 °C) and combined high pressure (up to 800 MPa)/thermal (20 up to 65 °C) treatments was studied on a kinetic basis. Residual folate concentration after thermal and high pressure/thermal treatments was measured using reverse phase liquid chromatography. The degradation of both folates followed first-order reaction kinetics. At ambient pressure, the estimated Arrhenius activation energy (E_a) values of folic acid and 5-methyltetrahydrofolic acid thermal degradation were 51.66 and 79.98 kJ mol^{-1}, respectively. It was noticed that the stability of folic acid toward thermal and combined high pressure thermal treatments was much higher than 5-methyltetrahydrofolic acid. High-pressure treatments at room temperature or higher (up to 60 °C) had no or little effect on folic acid. In the whole P/T area studied, the rate constant of 5-methyltetrahydrofolic acid degradation was enhanced by increasing pressure, and a remarkable synergistic effect of pressure and temperature on 5-methyltetrahydrofolic acid degradation occurred at temperatures above 40 °C. A model to describe the combined pressure and temperature effect on the 5-methyltetrahydrofolic acid degradation rate constant is presented.

KEYWORDS: Folates; pressure; thermal; kinetic; stability

INTRODUCTION

Folates exist in a large variety of foods including green leafy vegetables, fruits, meat products, beans, fermented dairy products, and cereals. It has been identified as one of the most important vitamins for normal human metabolic function.[1] Folate deficiency has become an important hypovitaminosis in daily consumption. Folate deficiency may cause megaloblastic anaemia (*1*), and it has a specific role in the prevention of neural tube defects (*2*).

Folates are sensitive to physical factors such as temperature, pressure, and exposure to light and thus can be affected during food processing. Several authors have reported evidence of the thermal degradation of folates, i.e., 5-methyltetrahydrofolic acid in buffer systems heated at temperatures up to 100 °C (*3*−*6*) and UHT conditions (*7*, *8*); folic acid in buffer systems (*9*); and folic acid and 5-methyltetrahydrofolic acid in food systems (*10*). It has been shown that thermal degradation kinetics of several folates in model systems is affected by pH and type of buffer ions (*11*). Wilson and Chen (*11*) have reported that the degradation kinetics of folic acid, 5-formyltetrahydrofolic acid, and 5-methyltetrahydrofolic acid at 100 °C followed first-order kinetics in a wide pH range (1−12). Folic acid and 5-formyltetrahydrofolic acid were found to be stable toward heating for 10 h at pH 4−12 and the stability decreased with decreasing pH lower than 4. The methyl folate derivatives showed the highest thermal stability at pH 7, and a rapid decrease in stability was observed under alkaline or acid conditions.

During the last two decades of high pressure research, it has been demonstrated experimentally that (i) (vegetative) microorganism inactivation; (ii) modification of biopolymers including enzyme (in)activation, protein denaturation and gel formation; (iii) quality retention (e.g., color, flavor, nutrition value); and (iv) modification of physicochemical properties of water can be obtained by a high pressure treatment at room temperature or moderately elevated temperature (*12*, *13*). High pressure processing at moderate temperature is currently being used commercially for the pasteurization of products such as fruit juice, guacamole, jam, oyster, and ham. Sterilization (inactivation of spores) requires combined high pressure with high temperature conditions and is currently not yet industrially exploited. Studies have shown that the vitamin content of fruit and vegetable products such as vitamins A, C, B_1, B_2, E, and folic acid could be maintained under combined high pressure

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and moderate temperature conditions applied for a short time period (5−10 min) (*14*, *15*).

So far, studies on the effects of high-pressure treatments on nutritional aspects of foods particularly folates are very limited and are mostly qualitative in nature. The use of high pressure in industrial applications requires the identification of optimal pressure/temperature/time combinations resulting in a limited quality degradation (including limited vitamin degradation) within the constraints set by the target microbial/spore inactivation. Hereto, the aim of this work was to study the stability of folic acid and 5-methytetrahydrofolic acid in model systems on a kinetic basis during combined high pressure and thermal processing in comparison with conventional thermal treatments. The advantage of using a model system instead of a real food system is a complete control of all different process parameters leading to more accurate data for model identification.

MATERIALS AND METHODS

Sample Preparation. A folic acid stock solution (1 mg/mL) was prepared by dissolving 10 mg of folic acid (>95% purity, $C_{19}H_{19}0_7N_6$, Sigma, MO) in 10 mL of sodium borate solution (0.05 M, pH 9.22), whereas a 5-methyltetrahydrofolic acid stock solution (1 mg/mL) was prepared by dissolving 10 mg of 5-methyltetrahydrofolic acid (>95% purity, Schricks Laboratory, Jona, Switzerland) in 10 mL of sodium borate solution (0.05 M, pH 9.22) with 0.4% β mercaptoethanol. The stock solutions were stored at −80 °C. Working solutions (folic acid of 5 μg/mL and 5-methyltetrahydrofolic acid of 10 μg/mL) were prepared daily in phosphate buffer (0.2 M, pH 7) for thermal or combined high pressure and thermal treatments. All organic solvents (methanol, acetonitrile) were obtained from Merck (Darmstadt, Germany). Milli-Q water was used to prepare all solutions. All procedures of sample preparation and all treatments were carried out under subdued light. Samples were covered with aluminum foil, avoiding direct contact with air, and all operations were carried out under nitrogen atmosphere whenever possible.

Thermal Treatment. Folic acid of 5 μg/mL and 5-methyltetrahydrofolic acid of 10 μg/mL in phosphate buffer (0.2 M, pH 7) were filled in capillary tubes (Hirschmann, $d = 1.5$ mm, and $L = 150$ mm) using a vacuum pump. The filled capillary tubes were sealed with flame on both sides. The samples were immersed in a water bath (60−90 °C) or in an oil bath (above 100 °C) during preset time intervals at constant temperature (isothermal conditions). To stop the thermal degradation, samples were immediately cooled in an ice bath after withdrawal. The residual concentration of folates was measured using HPLC (see further). The blank (A_o) was defined as the concentration of the non-heat treated sample.

Combined High Pressure−Temperature Treatment. Isobaric−isothermal degradation P/T experiments were conducted in a laboratory pilot scale, multivessel high-pressure apparatus (Resato, Roden, Netherlands) consisting of eight thermostated 8 mL pressure vessels which allow combining of high pressure (up to 1000 MPa) with temperature from 10 to 65 °C. The pressure transmitting fluid is an oil/glycol mixture (TR15, Resato). The working folic acid and 5-methyltetrahydrofolic acid solutions were contained in flexible microtubes (Elkay, 500 μL) while avoiding air bubbles in the tubes. The pressure was built up at a constant rate of 125−150 MPa/min and an equilibration period for 5 min (*16*) that allows temperature inside the vessels to evolve to its desired value was taken into account. Time = 0 was accounted after reaching the equilibration period. At this moment, the first pressure vessel was decompressed and the residual concentration of the corresponding vessel was considered as blank (A_o). The other vessels were then decompressed as a function of time. After withdrawal, the samples were stored in an ice bath and subsequently the residual vitamin concentration was measured.

HPLC Analysis. HPLC equipment (AKTA purifier, Amersham Biosciences, Uppsala, Sweden) was used to quantify the concentration of folic acid and 5-methyltetrahydrofolic acid. It was supported by Unicorn 4.0 software for data processing. Chromatographic separations were performed based on reversed phase chromatography using a Hypersil ODS C18 column (250 × 4.6 mm i.d., 5 μm particle size, Alltech, IL).

A gradient was performed using a mixture of acetonitrile-phosphate buffer (0.033 M, pH 2.15) with a flow rate of 1 mL/min. The gradient started at 5% (v/v) acetonitrile, which was maintained isocratically for the first 9 min (for folic acid) or 5 min (for 5-methyltetrahydrofolic acid), thereafter the acetonitrile concentration was raised linearly to 17% (v/v) within 30 min (for folic acid) or to 8.5% within 15 min (for 5-methyltetrahydrofolic acid). Both folates were detected using a UV detector at 290 nm. The column temperature was maintained at 25 °C and the injection volume was 100 μL. Folic acid and 5-methyltetrahydrofolic acid peaks were respectively found at retention time between 26 and 28 min and 16−17 min. The folate concentration was calculated based on their peak area and height in comparison to the internal standard solutions (in the range of 0−15 μg/mL) of the same components (dissolved in sodium borate solution (0.05 M, pH 9.22) with 0.4% β mercaptoethanol). The regression correlation (r^2) of the standard curves in this study was situated above 0.98.

Data Analysis to Determine Kinetic Parameters. Kinetic parameters of folate degradation after thermal and combined high pressure-thermal treatments were estimated as degradation rate constants (k), activation energies (E_a), and activation volumes (V_a). In general, an nth order degradation reaction can be presented as eq 1.

$$\frac{dC}{dt} = kC^n \tag{1}$$

where C is concentration of folates at treatment time t, k is the degradation rate constant, and n is the reaction order. Previous studies have shown that the thermal degradation of folates in buffer systems i.e., folic acid and 5-methyltetrahydrofolic acid followed first-order reaction kinetics ($n = 1$) in a wide pH range (1−12) (*1*, *6*, *11*). Therefore, under constant conditions and $n = 1$, eq 1 can be integrated to be eq 2.

$$\ln(C) = \ln(C_o) - kt^2 \tag{2}$$

where C_o is the concentration of folate at time = 0. When the natural logarithm of the residual concentration is plotted as a function of time, the degradation rate constant can be estimated by a linear regression analysis (*17*). The k value is derived from the slope of the regression line.

The temperature dependence of the k value is expressed as the activation energy (E_a) based on the Arrhenius relationship. In this investigation, the linearized form of this relationship (eq 3) was applied. This relation is valid at constant pressure.

$$\ln(k) = \ln(k_{refT}) + \left[\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right] \tag{3}$$

where k_{refT} is the degradation rate constant at reference temperature T_{ref}.

The pressure dependence of the k value is expressed as the activation volume (V_a) presented in eq 4 (a linearized form of Eyring equation). This relation is valid at constant temperature.

$$\ln(k) = \ln(k_{refP}) - \left[\frac{V_a}{RT}(P - P_{ref})\right] \tag{4}$$

where k_{refP} is the degradation rate constant at reference pressure P_{ref}.

RESULTS AND DISCUSSIONS

Thermal Degradation Kinetics of Folic Acid and 5-Methyltetrahydrofolic Acid. The effect of temperature on the folate degradation kinetics was studied in a temperature range from 65 up to 160 °C. By plotting the ln value of the relative residual concentration value [$\log(C/C_o)$] as a function of the treatment time at constant temperature, it can be observed that the kinetics of folic acid and 5-methyltetrahydrofolic acid

Table 1. Estimated Degradation Rate Constant ($\times 10^{-3}$ min^{-1}) of Folic Acid (5 μg/mL) and 5-Methyltetrahydrofolic Acid (10 μg/mL) Due to Thermal Treatment at Ambient Pressure

temp (°C)	folic acid	5-methyltetrahydrofolic acid
65	nd[b]	9.73 ± 0.92[a]
70	nd	13.06 ± 1.12
80	nd	28.14 ± 0.93
90	nd	68.31 ± 5.86
120	1.04 ± 0.19[a]	nd
140	1.26 ± 0.20	nd
160	4.73 ± 0.36	nd
E_a (kJ mol^{-1})	51.66 ± 25.40 $r^2 = 0.80$	79.98 ± 4.88 $r^2 = 0.993$

[a] Standard error of regression. [b] nd: not determined.

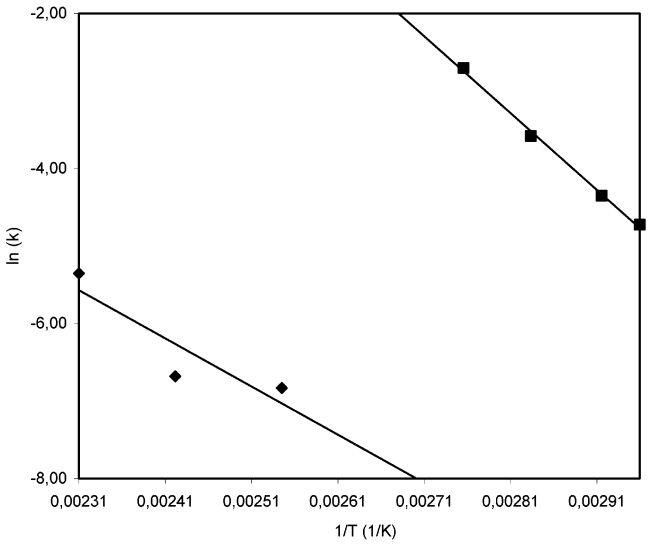

Figure 1. Temperature dependence of the degradation rate constant of folic acid (◆) and 5-methyltetrahydrofolic acid (■) in phosphate buffer (0.2 M, pH 7).

thermal degradation in phosphate buffer (0.2, pH 7) could be adequately described by a first-order kinetic model (eq 2). This is in line with previous reports (*1*, *6*, *11*). However, Ruddick and co-workers (*5*) reported that the degradation kinetic of 5-methyltetrahydrofolic acid in phosphate buffer (pH 7.3) under unlimited oxygen concentration followed a pseudo-first-order reaction. The estimated *k* values are summarized in **Table 1**.

The temperature dependence of folic acid and 5-methyltetrahydrofolic acid degradation rate constants at atmospheric pressure could be accurately described by a linearized Arrhenius relationship (eq 3) as illustrated in **Figure 1**. The estimated activation energy (E_a) of folic acid and 5-methyltetrahydrofolic acid was respectively 51.66 and 79.98 kJ mol^{-1}. In literature, the E_a values of 5-methyltetrahydrofolic acid thermal degradation have been reported by several authors. Values of 38.099 kJ mol^{-1} (*4*), 29.73 kJ mol^{-1} (*5*), and 106 kJ mol^{-1} (*8*) have been reported.

By comparing the heat stability of folic acid and 5-methyltetrahydrofolic acid, it could be concluded that 5-methyltetrahydrofolic acid was less thermostable than folic acid and the degradation rate constant of folic acid was less temperature dependent as compared to 5-methyltetrahydrofolic acid. Similar findings have also been observed by Wilson and Chen (*11*). The degradation of 5-methyltetrahydrofolic acid under alkaline condition in that study could be explained by an oxidative

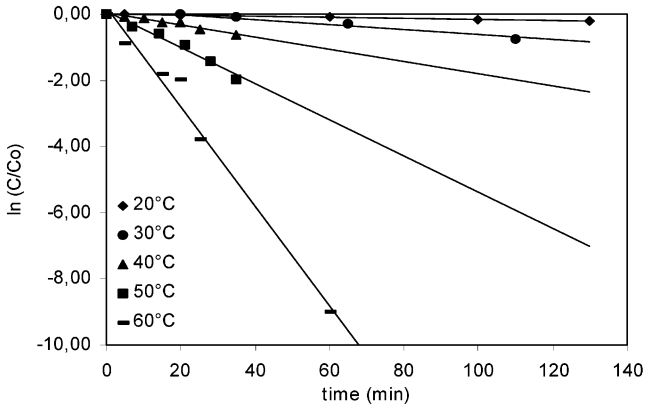

Figure 2. Isobaric isothermal degradation kinetics of 5-methyltetrahydrofolic acid (10 μg/mL) in phosphate buffer (0.2 M, pH 7) at constant pressure (800 MPa) and temperatures from 20 to 60 °C.

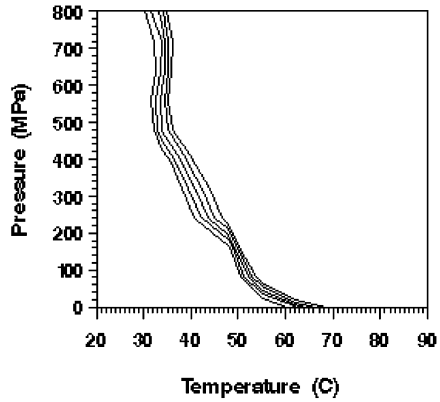

Figure 3. Pressure–temperature kinetics diagram for the P/T degradation of 5-methyltetrahydrofolic acid (10 μg/mL) in phosphate buffer (0.2 M, pH 7) (interpolated from experimental data). The inner and outer lines represent P/T combinations for *k*-value equal to 0.0075 and 0.0115 min^{-1}, respectively

reaction involving the formation of 5-methyldihydrofolic acid (*11*).

Pressure−Temperature Degradation Kinetics of Folic Acid and 5-Methyltetrahydrofolic Acid. Kinetics of isobaric isothermal degradation of folic acid and 5-methyltetrahydrofolic acid were studied in the pressure range from 100 to 800 MPa combined with temperatures from 20 to 65 °C. In the P/T area studied, it was observed that folic acid showed a higher stability than 5-methyltetrahydrofolic acid.

An extreme stability of folic acid toward pressure processing was observed, e.g., no reduction in folic acid concentration after a treatment at 600 MPa and 60 °C for 7 h. High-pressure treatments at room temperature or higher (up to 60 °C) resulted in no or little folic acid degradation.

The degradation of 5-methyltetrahydrofolic acid in phosphate buffer (0.2 M, pH 7) due to combined high pressure thermal conditions follows first-order kinetics (**Figure 2**). The estimated degradation rate constants of 5-methyltetrahydrofolic acid for several combinations of pressure and temperature are summarized in **Table 2** and an iso-degradation rate contour diagram of 5-methyltetrahydrofolic acid as function of pressure and temperature is depicted in **Figure 3**. These results show a synergistic effect of pressure and temperature on 5-methyltetrahydrofolic acid degradation at all temperature and pressure combinations tested. In the temperature and pressure range studied, a pressure increase at constant temperature enhanced the observed degradation. Likewise, a temperature increase at

Table 2. Estimated k-Values ($\times 10^{-3}$ min^{-1}) of 5-Methyltetrahydrofolic Acid (10 μg/mL) in Phosphate Buffer (0.2 M, pH 7) Due to Combined P/T Treatments[a]

P/T	20	30	40	50	60	65	70	80	90
0.1	nd[c]	nd	nd	nd	nd	9.73 ± 0.83[b] $r^2 = 0.96$	13.06 ± 1.12 $r^2 = 0.96$	28.14 ± 0.93 $r^2 = 0.99$	68.31 ± 5.86 $r^2 = 0.97$
100	nd	nd	nd	7.38 ± 0.47 $r^2 = 0.98$	24.21 ± 3.84 $r^2 = 0.91$	25.08 ± 3.43 $r^2 = 0.93$	nd	nd	nd
200	nd	nd	5.66 ± 1.04 $r^2 = 0.88$	11.74 ± 0.69 $r^2 = 0.99$	35.19 ± 1.69 $r^2 = 0.99$	38.12 ± 1.80 $r^2 = 0.99$	nd	nd	nd
400	nd	2.92 ± 0.34 $r^2 = 0.94$	11.03 ± 0.52 $r^2 = 0.99$	22.98 ± 8.29 $r^2 = 0.80$	70.12 ± 7.24 $r^2 = 0.95$	78.99 ± 8.86 $r^2 = 0.98$	nd	nd	nd
600	0.55 ± 0.096 $r^2 = 0.90$	4.51 ± 0.42 $r^2 = 0.97$	18.25 ± 1.60 $r^2 = 0.98$	25.96 ± 1.93 $r^2 = 0.98$	106.34 ± 17.20 $r^2 = 0.93$	nd	nd	nd	nd
800	1.60 ± 0.088 $r^2 = 0.99$	7.33 ± 1.20 $r^2 = 0.93$	18.32 ± 1.84 $r^2 = 0.95$	54.41 ± 4.22 $r^2 = 0.98$	150.37 ± 10.15 $r^2 = 0.98$	nd	nd	nd	nd

[a] Pressure in MPa, temperature in °C. [b] Standard error of regression. [c] nd: not determined.

Table 3. E_a-Values for Thermal Degradation of 5-Methyltetrahydrofolic Acid in Phosphate Buffer (0.2 M, pH 7) at Atmospheric and Elevated Pressures

pressure (MPa)	E_a (kJ mol^{-1})	r^2
0.1	79.98 ± 4.88	0.99
100	78.97 ± 24.16	0.91
200	71.88 ± 7.95	0.98
400	81.09 ± 5.39	0.99
600	100.18 ± 11.89	0.96
800	90.10 ± 3.30	0.99

[a] Standard error of regression.

constant pressure enhanced the pressure−temperature degradation of 5-methyltetrahydrofolic acid. In **Figure 3**, it can be seen that the same degradation rate constant can be achieved by selecting different pressure temperature combinations. The contour diagram for 5-methyltetrahydrofolic acid degradation shows a remarkable synergistic effect of pressure and temperature on 5-methyltetrahydrofolic acid degradation in phosphate buffer at temperatures above 40 °C.

By plotting the natural logarithm of the degradation rate constant as a function of the reciprocal temperature at constant pressure, activation energies of 5-methyltetrahydrofolic acid degradation at different pressure levels were obtained by a linear regression analysis. It was noticed that the linearized Arrhenius model (eq 3) was valid over the entire pressure and temperature domain studied. The estimated activation energies are summarized in **Table 3**. At constant pressure, the degradation rate constant of 5-methyltetrahydrofolic acid increased with increasing temperature. The estimated activation energy was situated between 71.88 to 100.18 kJ mol^{-1} in the pressure domain of 100 to 800 MPa. By increasing pressure, an increase in the estimated activation energy was observed (i.e., increase in thermosensitivity of the degradation rate constant). The highest thermosensitivity of the degradation rate constants was observed at pressure around 600−800 MPa. The influence of pressure on the degradation process at constant temperature can be analyzed using the Eyring model (eq 4) and the estimated values of activation volume (V_a) at constant temperature are presented in **Table 4**. At all temperature levels tested, the estimated activation volumes showed a negative sign, indicating an acceleration of the 5-methyltetrahydrofolic acid degradation by increasing pressure. The highest pressure stability of the degradation rate constants occurred at 30−40 °C. This means that applying a pressure treatment at temperature below 40 °C can relatively better maintain 5-methyltetrahydrofolic acid.

Formulation of Mathematical Model to Describe the Combined Pressure and Temperature Dependence of 5-Methyltetrahydrofolic Acid Degradation Rate Constant. On the basis of the kinetic data, it was tried to develop a model that could describe the combined pressure temperature dependence of the degradation rate constant of 5-methyltetrahydrofolic acid. At this moment, there is no general kinetic model available in the literature to describe the combined pressure−temperature effect on nutritional factors. Empirical mathematical models using the Arrhenius equation or the Eyring model as a starting point (*18*, *19*) or elliptical eq 20 have been used successfully as an approach to construct kinetic model describing pressure temperature inactivation of enzymes. These models were not appropriate to describe the combined pressure temperature dependence of the folate degradation rate constant (data not shown) because the standard error of all predicted kinetic model parameters was very high.

In literature, the thermodynamic model (*21*) has been applied to describe combined pressure and temperature dependence of enzyme inactivation (*22*−*25*) (eq 5). The thermodynamic model can be converted into a kinetic model (eq 8) using eq 6 and 7.

$$\Delta G = \frac{\Delta\beta}{2}(P - P_{ref})^2 + \Delta\alpha(P - P_{ref})(T - T_{ref}) - \Delta C_p\left[\left[T\left(\ln\left(\frac{T}{T_{ref}}\right) - 1\right)\right] + T_{ref}\right] + \Delta V_o(P - P_{ref}) - \Delta S_o(T - T_{ref}) + \Delta G_o \quad (5)$$

$$\Delta G^{\ddagger} = -R_g T \ln(K^{\ddagger}) \quad (6)$$

$$K^{\ddagger} = \frac{kh}{rk_B T} \quad (7)$$

$$\ln(k) = -\frac{\Delta\beta^{\ddagger}}{2R_g T}(P - P_{ref})^2 - \frac{\Delta V_o^{\ddagger}}{R_g T}(P - P_{ref}) + \frac{\Delta S_0^{\ddagger}}{R_g T}(T - T_{ref}) - \frac{\Delta\alpha^{\ddagger}}{R_g T}(P - P_{ref})(T - T_{ref}) + \frac{\Delta C_p^{\ddagger}}{R_g T}\left[T\left[\ln\left(\frac{T}{T_{ref}}\right) - 1\right] + T_{ref}\right] + \ln(k_{ref}) \quad (8)$$

It was examined whether the kinetic version of the thermodynamic model (eq 8) could describe the experimentally estimated 5-methyltetrahydrofolic acid pressure−temperature degradation rate constant. On the basis of the result of nonlinear regression analysis, the standard error of the estimated

Table 4. V_a Value for Pressure Degradation of 5-Methyltetrahydrofolic Acid in Phosphate Buffer (0.2 M, pH 7) at Elevated Temperatures

temp (°C)	V_a (cm^{-3} mol^{-1})	r^2
30	-5.79 ± 0.19[a]	0.99
40	-5.24 ± 1.39	0.90
50	-7.05 ± 0.88	0.96
60	-7.23 ± 0.67	0.94
65	-13.95 ± 2.49	0.94

[a] Standard error of regression.

Table 5. Estimated Values of Model Parameters Described in Eq 9 for Pressure and Temperature Degradation of 5-Methyltetrahydrofolic Acid in Phosphate Buffer (0.2 M, pH 7)

kinetic parameter	estimated value of eq 9
$\Delta V_0^{\ddagger}$ (cm^3 mol^{-1})	-8.86 ± 0.51[a]
$\Delta S_0^{\ddagger}$ (J mol^{-1} K^{-1})	249.80 ± 8.35
$\Delta\beta^{\ddagger}$ (cm^6 J^{-1} mol^{-1})	$(2.12 \pm 0.37) \times 10^{-2}$
$\Delta C_p^{\ddagger}$ (J mol^{-1} K^{-1})	-1258.60 ± 201.50
C	$(2.71 \pm 0.17) \times 10^{-2}$
corrected r^2	0.996
SD	0.273

[a] Asymptotic standard error.

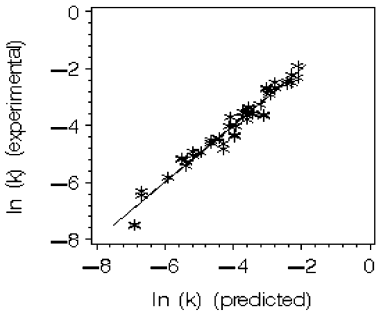

Figure 4. Correlation between the ln k_{obs} values determined from the experimental work and the estimated ln k_{obs} values using the model described in eq 9.

$\Delta\alpha^{\ddagger}$ was much higher than the estimated value. Hereto, in this case, the term $\Delta\alpha^{\ddagger}/R_gT$ was omitted and the reduced version of eq 8 (i.e., eq 9) was used to describe the combined pressure temperature dependence of the 5-methyltetrahydrofolic acid degradation rate.

$$\ln(k) = -\frac{\Delta\beta^{\ddagger}}{2R_gT}(P - P_{ref})^2 - \frac{\Delta V_o^{\ddagger}}{R_gT}(P - P_{ref}) + \frac{\Delta S_0^{\ddagger}}{R_gT}(T - T_{ref}) + \frac{\Delta C_p^{\ddagger}}{R_gT}\left[T\left[\ln\left(\frac{T}{T_{ref}}\right) - 1\right] + T_{ref}\right] + \ln(k_{ref}) \quad (9)$$

The estimated model parameters are summarized in **Table 5**. The relation between experimental and estimated k-values is illustrated in **Figure 4**. A satisfactory correlation between these values was found. By inserting the predicted model parameter values into eq 9, a predicted iso-degradation rate contour diagram of 5-methyltetrahydrofolic acid as a function of pressure and temperature was depicted (**Figure 5**). It could be seen that eq 9 can also predict the synergistic effect of pressure and temperature.

CONCLUSION

On the basis of the estimated degradation rate constants obtained in this study, it is obvious that folic acid in phosphate buffer (0.2 M, pH 7) has an enormous thermo and barostability as compared to 5-methyltetrahydrofolic acid. With respect to conventional thermal processing in industries, pasteurization (e.g., 71.7 °C/15 s or 62.7 °C/30 min) and sterilization (e.g., UHT processing (135 °C/1 s) or classical in-bottle sterilization (110−120 °C/10−20 min)) are mostly used for food preservation. During pasteurization, concentration of 5-methyltetrahydrofolic acid will be slightly lost about 10−15% of the initial concentration (recalculated based on the *k*-values in **Table 1**), whereas 5-methyltetrahydrofolic acid will be totally destroyed by sterilization. In contrary to folic acid, its initial concentration can be kept even after sterilization. Regarding to the use of high-pressure technology in commercial pasteurization, pressurization (lower than 800 MPa) at moderate temperatures (lower than 40 °C) for a short period (less than 30 min) allows to maintain the 5-methyltetrahydrofolic acid concentration while applying pressure at high temperatures (above 40 °C) can promote an increase in 5-methyltetrahydrofolic acid degradation. In this study, an adequate model to describe the combined pressure and temperature dependence of 5-methyltetrahydrofolic acid degradation rate constant has been formulated based on the model described by Hawley (*21*). Hereto, optimal pressure/temperature/time combinations can be identified for process optimization.

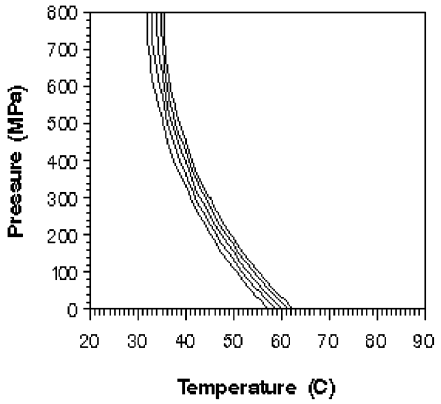

Figure 5. Predicted pressure–temperature kinetic diagram for P/T degradation of 5-methyltetrahydrofolic acid (10 μg/mL) degradation in phosphate buffer (0.2 M, pH 7), by inserting the estimated model parameters into eq 9. The inner and outer lines represent *k* values equal to 0.0075 and 0.0115 min^{-1}, respectively.

In this investigation, the stability of folates, i.e., folic acid and 5-methyltetrahydrofolic acid, during thermal and combined high pressure/thermal treatments has been studied in a model system. Interpreting stability of folic acid and 5-methyltetrahydrofolic acid in real food systems (even at the same pH) must be made with cautions since existences of natural (anti)oxidants can give influences on the stability of folates and the concentration of folic acid and 5-methyltetrahydrofolic acid in native foods is lower than in the model system.

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