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Kinetics of chemical marker formation in whey protein gels for studying microwave sterilization

M.H. Lau [a,1], J. Tang [a,*], I.A. Taub [b], T.C.S. Yang [b], C.G. Edwards [c], R. Mao [a,2]

[a] *Department of Biological Systems Engineering, Washington State University, 213 L J Smith Hall, Pullman, WA 99164-6120, USA*

[b] *Advanced Processes Team, Combat Feeding Program, US Army Soldier and Biological Chemical Command, Natick Soldier Center, Natick, MA 01760, USA*

[c] *Department of Food Science and Human Nutrition, Washington State University, Pullman, WA 99164-6376, USA*

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Abstract

The kinetics of 4-hydroxy-5-methy-3(2H)-furanone (M-2) formation in a model food system (20% whey protein gel) was determined for studying cumulative time–temperature effects in high-temperature-short-time processes. M-2 was formed from D-ribose and amines through non-enzymatic browning reactions and enolization under low acid conditions (pH > 5). The order of the reaction for M-2 formation was determined by non-linear regression analysis and further confirmed by graphical method. M-2 formation followed a first-order kinetics and the rate constant temperature dependence was described using an Arrhenius relationship. The reaction rates and activation energy were determined using two-step, multi-linear and non-linear regression analyses. This study also demonstrated the use of M-2 formation in determining the cumulative heating effect in a model food system subjected to 915 MHz microwave heating.

Keywords: Kinetics; Chemical marker; Whey protein; Ribose; Microwave; Thermal processing; Validation method

1. Introduction

High-temperature-short-time (HTST) processes have been developed for liquid foods to reduce the adverse thermal degradation in food qualities while ensuring food safety (Lund, 1977). The possibility of using HTST processes for solid foods by conventional methods is, however, limited by the slow heat conduction which often causes overheating at the solid surface during the time needed for the heat to be transferred from the heating medium to the slowest heating point of the food (Lund, 1977; Meredith, 1998). Microwave heating may overcome the limitation imposed by the slow conventional heating (Meredith, 1998). The volumetric heat generated by microwaves can significantly reduce the total heating time at the elevated temperatures needed for commercial sterilization (Decareau, 1985). However, microwave sterilization processes have not been used in the US due to several possible reasons, including (1) often unpredictable and uneven microwave energy distribution within the product, (2) the difficulty in monitoring and predicting microwave heating pattern in the microwave cavity as well as within the food product. Before microwave sterilization can be approved by FDA and accepted by the US food industry, there are two issues that need to be addressed: (1) development of a system that can deliver predictable and uniform heating to food systems, and (2) development of a reliable monitoring procedure to ensure the safety of microwave processed foods (US Department of Health and Human Services, 2000).

In order to design an effective thermal process to ensure adequate sterility for shelf-stable foods, it is necessary to determine the location of the "cold-spot" in packaged foods (CFR 21, Part 113, 1983). For conventional thermal processes, heat is transferred from the heating medium (steam or water) to food interior via conduction (solid foods) or convention (liquid foods),

* Corresponding author. Tel.: +1-509-335-2140; fax: +1-509-335-2722.

E-mail address: jtang@mail.wsu.edu (J. Tang).

[1] Current Address: Kraft Foods Technical Center, 801 Waukegan Road, Glenview, IL 60025, USA.

[2] Current Address: Indium Corporation of America, 1676 Lincoln Avenue, Utica, NY 13323, USA.

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Nomenclature

A	molar concentration of the ribose	M	marker yield
A_0	initial molar concentration of ribose	m	reaction order to AA
AA	amino acid	M_∞	final marker yield
B	molar concentration of the AA	M_0	initial marker yield
B_∞	final molar concentration of AAs	n	reaction order to ribose
B_0	initial molar concentration of AAs	R	universal gas constant 1.987 cal/mol K
E_a	activation energy, kcal/mol	T	temperature, K
k	reaction rate constant	t	time, min
k_{ref}	reaction rate constant at 123.5 °C	T_{ref}	reference temperature at 123.5 °C

and the coldest location in the package is well defined, e.g., normally at the geometrical center in solid foods or about 1/5 from the bottom of the cans for liquid foods (Holdsworth, 1997). A commercial thermal process is designed based on measured temperature history of the cold spot in packaged foods in a retort. However, microwave heating is different from conventional heating in which the heating patterns usually depend upon the direct interaction between microwave energy and food and are difficult to predict (Decareau, 1985). Thus, assessment of temperature distribution within the packaged foods during microwave sterilization is essential, but yet it cannot be determined with single-point or even various-point temperature measurements (Ohlsson, 1972). Similar difficulty has been experienced in other advanced thermal processing methods, including ohmic heating and aseptic processing of liquid food containing large particulates. These have stimulated the development of a chemical marker method as an alternative evaluation tool to determine the heat distribution within the product during sterilization processes (Kim, Taub, Choi, & Prakash, 1996a, Chap. 6).

A chemical marker method was developed at the United States Army Natick Research Center (Kim & Taub, 1993). Three markers have been identified in various food systems, they are 2,3-dihydro-3,5-dihydroxy-6-methyl-(4H)-pyran-4-one (commonly referred to as M-1), 4-hydroxy-5-methy-3(2H)-furanone (M-2) and 5-hydroxymethylfurfural (M-3) (Kim & Taub, 1993). Kim and Taub (1993), Kim et al. (1996b) and Ramaswamy, Awuah, Kim, and Choi (1996) have used the M-1 yield in meat products as a temperature–time integrator in ohmic heating and aseptic processing. More recently, the yield of M-2 in whey protein gels as model foods has been effectively used by Prakash, Kim, and Taub (1997), Yang, Yang, and Taub (1998) and Lau (2000) and to quantitatively assess heating uniformity of microwave heating at 915 or 2450 MHz. However, a lack of kinetics information for M-2 prohibited researchers from quantitatively relating the chemical marker yields to time–temperature effect in microwave sterilization process.

Correct prediction of the microwave power delivered to the food systems using chemical marker information relies on the accuracy of kinetics parameters, which, in turn, is greatly dependent on the experimental data collection and the parameter estimation techniques. The most common method used to estimate the Arrhenius parameters is the classic two-step linear regression method (Lund, 1983). Several researchers, however, considered the two-step linear regression analysis to be the least accurate because it estimates too many intermediate values (Cohen & Saguy, 1985; Haralampu, Saguy, & Karel, 1985; Van Loey, Fransis, Hendrickx, Maesmans, & Tobback, 1995). In order to avoid some of the drawbacks of the classic two-step linear regression method, multi-linear and non-linear regression methods have been suggested (Haralampu et al., 1985; Lund, 1983). According to Haralampu et al. (1985), multiple linear regression gives a more accurate estimation of rate constant at each temperature than the classic two-step linear regression method because the multiple linear regression analysis uses a single initial reactant concentration for all data points. However, the estimated Arrhenius parameters could be biased when using multiple linear regression analysis because the reaction rate estimates were not independent, thus violating the assumption for least squares regression (Haralampu et al., 1985). Arabshahi and Lund (1985) and Cohen and Saguy (1985) recommended a one step non-linear regression method, because the regression can be carried out to fit all data, thus, substantially increasing the degrees of freedom and giving much narrower confidence intervals for the estimated parameters (Chung & Merritt, 1991; Cohen & Saguy, 1985). Van Loey et al. (1995), however, pointed out that the estimated kinetic parameters from non-linear regression analysis can also be biased because it is possible to converge at a local minimum of the error mean squares function. In order to accurately estimate the Arrhenius parameters, some studies used two to three different regression methods to process experimental data (Chung & Merritt, 1991; Lund, 1983; Cohen & Saguy, 1985; Van Loey et al., 1995).

The objective of this study was to determine the reaction order, rate constants and activation energy for the M-2 formation in whey protein gels at sterilization temperatures. We evaluated three regression methods in the determination of the Arrhenius parameters. We also used the obtained parameters to predict the M-2 yields for a 915 MHz microwave heating sterilization process and compared the predicted values with the experimentally determined M-2 yield.

2. Theory

2.1. Mechanism of the marker (M-2) formation

During a heating process, several amino acids (AAs), particularly lysine, arginine, histidine and methionine, may interact with glucose or ribose through Maillard (non-enzymatic browning) reactions to form Amadori compounds (Whistler & Daniel, 1985; Yaylayan, Fichtali, & van de Voort, 1994). In the formation of Amadori compounds, 1,2-enolization is favored in acidic media (pH < 4), where the nitrogen atom of the Amadori compound is protonated (Kim et al., 1996a, Chap. 6). Subsequent dehydration leads to formation of 2-furaldehyde from ribose (Fig. 1) (Kim et al., 1996a, Chap. 6). Under weak acidic (pH > 5) or alkaline (pH > 7) conditions, 2,3-enolization leads to the formation of furanones (M-2 compound) (Feather, 1981; Kim et al., 1996a, Chap. 6). In the experiment conducted by Kim et al. (1996a, Chap. 6), formation of 2-furaldehyde dominated at low pH conditions and M-2 formation became more important at pH above 4.5 using 1% D-ribose added to beef extract. M-2 compound was identified by Kim, Ball, Giles, and White (1994) using mass spectrometry. An anion exclusion chromatographic separation and photodiode array detection showed that

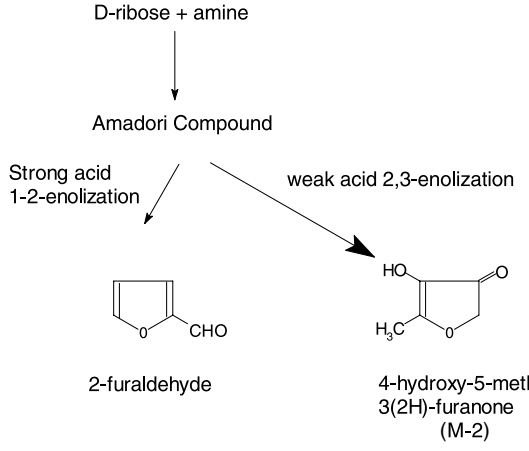

Fig. 1. Summary of reaction pathways leading to the chemical marker formation (Kim et al., 1996a, Chap. 6).

M-2 elutes with 5.6 min retention time and has a UV absorption maximum at 285 nm (Kim & Taub, 1993).

2.2. Theoretical considerations for kinetic modeling of M-2 formation

In whey protein gels containing 1% ribose, the concentration of ribose is much smaller than that of AAs. The chemical reaction of M-2 formation can be represented as:

$$\begin{array}{lccccc} & \text{Ribose} & + & \text{AA} & \rightarrow & \text{M-2} \\ t=0 & A_0 & & B_0 & & M_0 \\ t=t & A & & B & & M \\ t=\infty & 0 & & B_\infty & & M_\infty \end{array} \quad (1)$$

where A, B, and M are the molar concentrations of the reactants ribose, amines, and product M-2 respectively. As the reaction progresses, A, B, and M change continuously with time until all ribose is consumed. For reaction (1), the reaction rate can be generally written as (Adamson, 1973):

$$\frac{dM}{dt} = k_{\text{total}} A^n B^m \quad (2)$$

That is, the reaction is nth order to ribose, mth order to AA, and the total order of reaction is $n+m$. Under the condition $A_0 \ll B_0$, very small fraction of AA is used during the reaction and B is essentially constant. Eq. (2) is then simplified into:

$$\frac{dM}{dt} = kA^n \quad (3)$$

where

$$k = k_{\text{total}} B_0^m \quad (4)$$

Thus, reaction (1) becomes a pseudo-nth-order reaction. Based on the material balance in reaction (1), we have:

$$A_0 + M_0 = A + M = M_\infty \quad (5)$$

and, therefore, Eq. (3) becomes:

$$\frac{dM}{dt} = k(M_\infty - M)^n \quad (6)$$

3. Materials and methods

3.1. Selection of a model food system

When developing a model food system for chemical markers, the kinetic parameters determined from experiments depend on the raw materials. Whey protein concentrate (WPC) was selected in this study because it has the specific type of AAs needed for M-2 compound formation. In addition to the AAs availability, WPC can be easily dispersed in water and formed firm gels at 80 °C which would not cause any M-2 formation. Since the

WPC can be easily dispersed in water, the substrate (AAs) would be equally distributed throughout the whey protein gel system. WPC is readily available commercially and has a low cost. Preliminary experiments showed that the whey protein gels possessed a strong water-holding capacity during high temperature heating. The minimum losses of water from the whey protein gels can prevent the migration of marker within the gel system. Based on all the criteria mentioned above, WPC would be a suitable candidate for chemical marker studies.

3.2. Thermal treatments of whey protein gels in capillary tubes

For the preparations of whey protein gel, 20 g WPC (Alacen 882, containing 80% protein on dry basis, Santa Rosa, CA) and 1 g of ribose (Sigma, St. Louis, MO) were dispersed in 79 g of deionized water at 20 °C. About 2 ml of the dispersed solution were then carefully injected into the middle section of capillary tubes (inner diameter = 1.5 mm; outer diameter = 1.55 mm; length = 15 mm). Both ends of the tube were sealed with hot flame. During the sealing of tubes, precaution was taken in order to avoid heating in the whey solution. The transition temperature for WPC in 0.7 M phosphate buffer at pH 6.0 was between 68 and 83 °C (Palumbo, 1992). Preliminary studies have shown that the 20% WPC solution formed a firm gel at 80 °C with 40 min heating with a minimum marker yield (<8%). This temperature–time combination was used to set the gel in the capillary tubes using water baths. The reason for forming the whey protein gel is because in temperature uniformity testing using chemical market analyses, whey protein solution was first formed into gel and then cut into the required shape. Therefore, in our kinetic studies, it is important to form the whey protein gel containing ribose before exposing the gel to desired heat treatment.

The kinetic studies were carried out at 116, 121, 126 and 131 °C (in an oil bath) with different time intervals in order to cover possible temperature–time combinations in microwave sterilization processes. After heating, the tubes containing whey protein gels were immediately removed from the heating bath at the end of each time interval and plunged into an ice water bath for rapid cooling. Extraction of marker compound was carried out immediately after the cooling process. The kinetic experiments were carried out in duplicate.

3.3. HPLC analysis

A HPLC system was used to analyze M-2 yield in whey protein gels after the heat treatments. The system consisted of a photodiode array detector (Hewlett–Packard 1040A, Plainsboro, NJ) and a solvent delivery system (ISCO model 2350, Lincoln, NE) controlled by a desktop computer and connected to an integrator. To prepare samples for HPLC analysis, heated whey protein gels were taken out of capillary tubes and weighed. The samples were then homogenized with 4 ml of 10 mM H_2SO_4, centrifuged and filtered through 0.45 μm nylon membrane filters. The filtered solutions were then injected into an anion exclusion column (Bio-RAD, Hercules, CA) equipped with an automatic injection system (Hewlett–Packard 1050 Series, Plainsboro, NJ). The mobile phase used was 10 mM H_2SO_4 at a flow rate of 1 ml/min. Absorbance of the effluent was determined at 285 nm as per Kim and Taub (1993). Results (M-2 yield) from the HPLC analysis were presented as the peak area observed divided by the total mass of the gel.

4. Data analysis

4.1. Determination of the reaction order for M-2 formation

The reaction order of the M-2 formation was determined by non-linear regression analysis. A graphical method was used to verify the result.

4.1.1. Non-linear regression analysis

The non-linear regression analysis was based on the integrated form of Eq. (6):

$$M = M_\infty - \left[(M_\infty - M_0)^{1-n} - (1-n)kt\right]^{1/(1-n)} \quad (n \neq 1) \tag{7}$$

The non-linear regression procedure of Sigma Plot (SPSS Inc., 1997) was used to estimate the value of n from experimentally determined M versus time relationship. Values for M_0, and M_∞ were also estimated by the non-linear fitting of Eq. (7) to experimental data at each temperature.

4.1.2. Graphical analysis

A linear regression analysis was performed on the plot of $\ln(M_\infty - M)$ versus time for the first-order reaction or $(M_\infty - M)^{1-n}$ versus time for other orders of reactions. The best fitted straight line was decided by examining the coefficient of determination (r^2) (Hill & Grieger-Block, 1980).

4.2. Kinetics parameters determination

Once the order of reaction for M-2 formation was determined by the non-linear regression analysis and confirmed by the graphical method, the kinetic parameters were determined by modified two-step, multi-linear and one-step non-linear regression methods.

4.2.1. Modified two-step regression

For first order reaction, Eq. (6) becomes:

$$\frac{dM}{dt} = k(M_\infty - M) \tag{8}$$

Integrating Eq. (8) yields:

$$-\ln(M_\infty - M) = kt - \ln(M_\infty - M_0) \tag{9}$$

Rearranging Eq. (9) yields:

$$M = M_\infty - (M_\infty - M_0)\exp(-kt) \tag{10}$$

The Arrhenius relationship for the effect of temperature on the rate constants of marker formation can be expressed below:

$$k = k_{\text{ref}} \exp\left(-\frac{E_a}{R}\left[\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right]\right) \tag{11}$$

where k_{ref} is the rate constant at the reference temperature T_{ref} (K), E_a is the activation energy and R is the universal gas constant which is 1.987 cal/mol K. The T_{ref} in this study was taken as 123.5 °C or 396.8 K, the mean of the experimental temperature range. The logarithmic form of Eq. (11) is:

$$\ln k = \ln k_{\text{ref}} - \frac{E_a}{R}\left[\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right] \tag{12}$$

Normally, the classic two-step linear regression method was used to estimate the Arrhenius parameters by first regressing the logarithmic reactant concentration versus time at each temperature to estimate the rate constant (k) and then regressing $\ln(k)$ versus $1/T$ to obtain $\ln(k_0)$ and activation energy (E_a). In the current study, the two-step method was done slightly differently, since the M_∞ in Eq. (9) needs to be known prior to the determination of k. The Marquardt–Levenberg algorithm for non-linear regression was used to obtain the M_∞ and k at each temperature. Kinetic activation energy was obtained by linear regression on the rate constants versus temperatures.

4.2.2. Two-step multi-linear regression

For the first-order reactions, k_i values at different temperature T_i were first obtained by multi-dimensional linear regression using:

$$-\ln(M_\infty - M) = \sum_{i=1}^{m} k_i t_{T_i} - \ln(M_\infty - M_0) \tag{13}$$

where t_{Ti} is modified time variable. Detailed information on using Eq. (13) is provided in Haralampu et al. (1985).

For the same reason as two-step linear regression, i.e., without the prior knowledge of M_∞, the two-step multi-linear regression method can not be completed independently in this study. To solve this problem, the M_∞ value obtained from non-linear regression method was used in calculating $\ln(M_\infty - M)$. In this manner, the two-step multi-linear regression method was used and k values at four different reaction temperatures were obtained by fitting the experimental data to Eq. (13). After all the k values at different reaction temperatures were obtained, then, it is straight forward to fit rate constant versus temperature data in the following equation by linear regression:

$$\ln k_i = \ln k_{\text{ref}} - \frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right) \tag{14}$$

4.2.3. One-step non-linear regression

Substituting k in Eq. (10) with Eq. (11), we obtain:

$$M = M_\infty - (M_\infty - M_0) \times \exp\left\{-tk_{\text{ref}} \exp\left[-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)\right]\right\} \tag{15}$$

The non-linear regression procedures in Sigma Plot (SPSS Inc., 1997) were used to fit the marker yield (M) versus time (t) data to Eq. (15).

5. Results and discussion

The chromatogram of M-2 formation from the HPLC analysis is shown in Fig. 2. The M-2 compound had an elution time of 5.6 min. This agrees with that reported by Kim and Taub (1993) who used the same sample extraction methods and HPLC conditions.

5.1. Estimation of the reaction order

Table 1 summarizes the results of the non-linear regression method to determine the reaction order. The results suggest that reaction expressed in Eq. (1) is a pseudo-first-order reaction. An ANOVA test showed no significant difference ($p > 0.05$) among the M_∞ from all heating temperatures. In other words, M_∞ is independent of heating temperatures. Although M_0 data was experimentally determined as 836 ± 40 peak area/g, it was not used in Eq. (7). Instead, the M_0 in Eq. (7) was treated as an unknown variable and was estimated by the non-linear regression process. The non-linear regression results show that the estimated M_0 values (Table 1) agree with the experimental data. Examples of the non-linear regression fitting are shown in Fig. 3. The

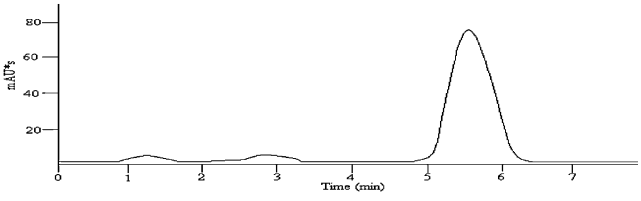

Fig. 2. The chromatogram of M-2 formation at 285 nm from HPLC analysis.

Table 1
Estimated order of reaction (n), M_0 and M_∞ values by non-linear regression

T (°C)	n	M_0 (peak area/g)	M_∞ (peak area/g)	r^2
116	0.95 ± 0.13	817 ± 171	10 700 ± 300	0.998
121	0.84 ± 0.10	812 ± 178	10 500 ± 400	0.997
126	1.04 ± 0.20	786 ± 132	11 500 ± 700	0.999
131	0.77 ± 0.19	873 ± 119	10 900 ± 200	0.999

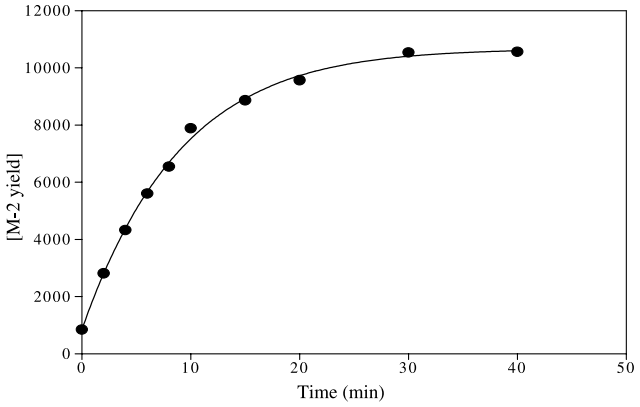

Fig. 3. Results from non-linear regression to determine the reaction order.

results from graphical determination of reaction order are summarized in Table 2. Once again, the values of r^2 indicated a first-order reaction for M-2 formation. A first-order plot, i.e., plot of $\ln(M_\infty - M)$ versus time, is shown in Fig. 4.

A first-order reaction was also obtained by Kim and Taub (1993) for the formation of M-1 in broccoli ex2-tract. Labuza and Baisier (1992) indicated that if amines or reducing compounds are at much more excessive levels in the non-enzymatic browning reaction, the loss of the limiting reactant can be modeled as a pseudo-first-order reaction.

5.2. Determination of the rate constants and activation energy

Based on the n value from non-linear and graphics analyses, a first-order kinetics for the M-2 formation was used to calculate the reaction rate constants as well as the activation energy. The reaction rate constants and

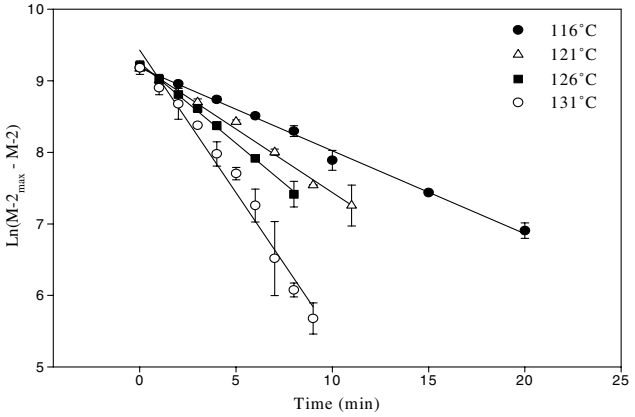

Fig. 4. Formation of M-2 followed a first-order reaction kinetics.

activation energy for M-2 formation were obtained by two-step, two-step multi-linear and one-step non-linear regression methods. The results of these regression analyses are given in Table 3. In contrast to two-step and the two-step multi-linear methods, the k_{ref} and E_a obtained by the non-linear method were directly calculated from fitting Eq. (15) to the original experimental data without first estimating k at each temperature. The fitting is shown in Fig. 5. The rate constant k at each temperature was calculated back from the k_{ref} and E_a (Table 3). The standard error in k, shown in Table, was estimated by:

$$\Delta k = k\left[\left|\frac{\Delta k_{ref}}{k_{ref}}\right| + \left|\frac{\Delta E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right|\right] \tag{16}$$

The values of r^2 and the estimated errors for E_a indicate that the one-step nonlinear method is the best among the three methods, followed by the two-step method. The multi-linear regression yielded the smallest r^2 and the largest standard error of estimation for the activation energy.

A review of activation energy for the non-enzymatic browning on model food systems was given by Labuza and Baisier (1992), where the activation energies ranged from 16 to 30 kcal/mol. The activation energy obtained in the current study for M-2 formation (19.48 kcal/mol, from the one-step non-linear method) is within the range cited in the literature for non-enzymatic browning (Labuza & Baisier, 1992). It needs to be noted that the method of data analysis affects kinetic parameters.

Table 2
Estimation of the order of M-2 formation by examining the r^2 from plots of zero-, half-, first- and second-order reactions

Temperature (°C)	Zero order $(M_\infty - M)$ versus time	Half order $(M_\infty - M)^{0.5}$ versus time	First order $\ln(M_\infty - M)$ versus time	Second order $1/(M_\infty - M)$ versus time
116	0.752	0.962	0.995	0.923
121	0.559	0.994	0.998	0.676
126	0.837	0.975	0.977	0.935
131	0.900	0.989	0.994	0.754

Table 3
Rate constants, k (min^{-1}), and activation energy, E_a (kcal/mol), for M-1 and M-2 formation at four temperatures

Reaction rate activation energy	Two-step regression	Multiple regression, M-2 formation	Non-linear regression	Two-step regression, M-1 formation[a]
$k_{116\ °C}$	0.112 ± 0.005	0.089 ± 0.008	0.110 ± 0.005	0.0099
$k_{121\ °C}$	0.166 ± 0.011	0.094 ± 0.008	0.152 ± 0.006	0.0146
$k_{126\ °C}$	0.190 ± 0.008	0.238 ± 0.018	0.207 ± 0.007	0.0213
$k_{131\ °C}$	0.243 ± 0.011	0.311 ± 0.021	0.281 ± 0.013	0.0309
k_{ref}	0.172 ± 0.006	0.159 ± 0.020	0.178 ± 0.005	0.0198
E_a (kcal mol^{-1} K^{-1})	15.29 ± 2.09 ($r^2 = 0.964$)	29.22 ± 7.16 ($r^2 = 0.893$)	19.48 ± 0.76 ($r^2 = 0.995$)	23.7

[a] Kinetics information was obtained from broccoli extract (Kim & Taub, 1993).

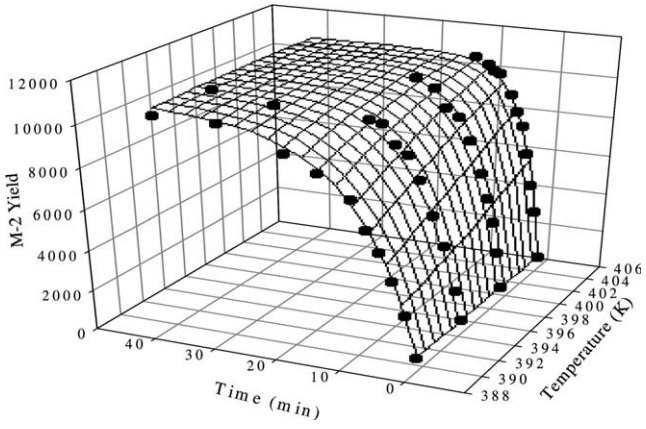

Fig. 5. One-step non-linear regression fitting Eq. (15) to experimental data.

Therefore, it is very important that a reliable method be used in determining parameters for kinetic models.

Table 3 compares the reaction rate constants for M-2 with the published M-1 reaction rates from Kim and Taub (1993). The reaction rate constants for formation of M-2 in whey protein gels were about 10 times larger than those of M-1 formation in broccoli extract (Kim & Taub, 1993). The higher k value for M-2 suggests that M-2 formation might be more useful in HTST study and M-1 might find more use in longer heating processes which often take more than 30 min or so. For example, in a HTST microwave sterilization process for a total cooking time of 3.5 min at 128 °C ($F_0 = 6$ min) (Ohlsson, 1991), the (M/M_∞) ratio was 0.5 for M-2 and 0.04 for M-1. However, in a conventional canning process for a total cooking time of 45 min with the retort temperature at 121 °C ($F_0 = 6$ min) (Ohlsson, 1991), the (M/M_∞) ratio was 0.95 (close to the saturation point) for M-2 and 0.22 for M-1. In the chemical marker method, it is desirable to limit the M/M_∞ ratio in an approximately linear range (e.g., $M < 5000$ unit in Fig. 3, or ~ 0.45 M/M_∞ ratio) to provide adequate sensitivity.

The activation energy for M-2 formation obtained from the current study (19.48 kcal/mol from the one-step non-linear method) is different from the activation energy of M-1 (23.7 kcal/mol) obtained from Kim and Taub (1993) (Table 3). According to Ross (1993), one needs two chemical markers with different formation activation energies to be able to estimate microbial destruction in a thermal process based on the yields of the two markers. There is, therefore, possible to use M-1 and M-2 to determine the lethality of a thermal process.

6. Validation of kinetic parameters

In order to evaluate the accuracy of the determined E_a and k_{ref} for M-2 formation in predicting the cumulative effect of microwave heating in foods, experiments were conducted using 20% whey protein gels (6 cm × 12 cm × 2 cm) containing 1% ribose heated in a pressurized 915 MHz microwave system described in Guan, Plotka, Clark, and Tang (2002). Processing time and temperature at the geometric center of the whey protein gels were recorded using a small (1 mm diameter) photonetic fiber optical sensor (Photonetics, Inc., Peabody, MA) that did not interfere with electromagnetic field in the measured sample. After heating, a small portion of the

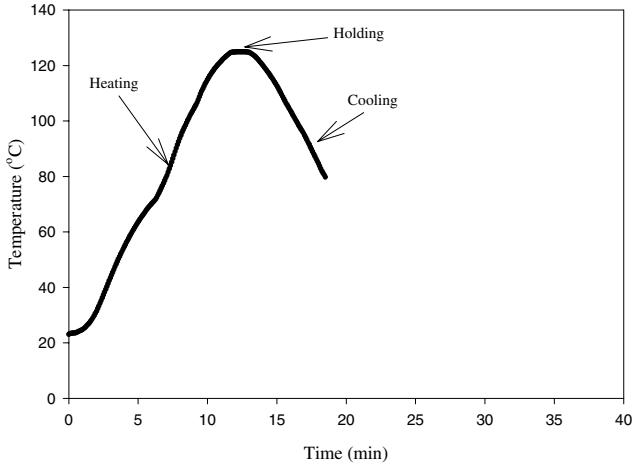

Fig. 6. A typical temperature history of whey protein gel (containing 1% ribose) heated in a 915 MHz pilot scale pressurized microwave system.

Table 4
Comparison of predicted and measured M-2 yield (M/M_∞) after heating in a pilot scale 915 MHz microwave sterilization unit

Cumulative lethality, F_0 (min)	Measured	Predicted using kinetics parameters		
		$E_a = 15.29$ kcal/mol $k_{121\ ^\circ C} = 0.166$ min^{-1} from two-step regression	$E_a = 29.22$ kcal/mol $k_{121\ ^\circ C} = 0.094$ min^{-1} from multi-linear regression	$E_a = 19.48$ kcal/mol $k_{121\ ^\circ C} = 0.152$ min^{-1} from non-linear regression
3.2	0.58 ± 0.05	0.57	0.46	0.54
4.9	0.63 ± 0.03	0.66	0.56	0.64

whey protein gel located near the fiber optical sensor was cored and then divided into top and bottom sections (radius = 0.5 cm, height = 1 cm) for evaluating M-2 yield. The following equations were developed to predict the marker formation based on the measured temperature–time history and pre-determined E_a and k_{ref}.

Substituting Eq. (11) into Eq. (8) yields:

$$\frac{dM}{dt} = k_{ref} \exp\left(-\frac{E_a}{R}\left[\frac{1}{T} - \frac{1}{T_{ref}}\right]\right)(M_\infty - M) \tag{17}$$

Integrating Eq. (17) gives:

$$M(t) = M_\infty - (M_\infty - M_0) \times \exp\left[\int_0^t -k_{ref} \exp\left(-\frac{E_a}{R}\left[\frac{1}{T} - \frac{1}{T_{ref}}\right]\right)dt\right] \tag{18}$$

The predicted M-2 formation was calculated by numerical integration of the right side of Eq. (18) using time–temperature data obtained from the 915 MHz pressurized microwave treatments (Fig. 6). The time interval for conducting the numerical integration was 10 s. The predicted M-2 yields based on the kinetic parameters obtained from the two-step linear regression method and the one-step non-linear regression methods agree well with the experimental values for the tested two levels of process lethality ($F_0 = 3.2$ and 4.9) (Table 4). The M-2 prediction based on the kinetic parameters obtained with the two-step multi-linear regression did not agree with the measured value for both process conditions. Therefore, the kinetic parameters obtained from non-linear regression and the two-step linear regression methods can be used in the future studies of using M-2 as time temperature indicator in thermal processes.

7. Conclusion

A pseudo-first-order reaction was obtained for M-2 formation. Reaction rates and activation energy obtained by the one-step non-linear regression analysis and a two-step non-linear regression method are more accurate than those obtained from a two-step multi-linear regression method. The kinetic parameters obtained from above two methods can be used in future studies of HTST microwave sterilization processes.

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