Kinetics of chemical marker M-1 formation in whey protein gels for developing sterilization processes based on dielectric heating

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Abstract

Determining the cold-spot in packaged foods is a critical step to ensure commercial sterility of the processed products in developing novel thermal processes. In dielectric heating (i.e. heating with microwave and radio frequency energy), the cold-spot is different from that of conventional heating. Chemical marker methods can be used to map heating patterns within foods systems. This study determined the kinetic parameters of the M-1 formation in whey protein gels at sterilization temperatures. Pre-formed whey protein gels containing 2% glucose sealed in capillary tubes were heated for different time intervals in oil baths at 116, 121, 126 and 131°C. M-1 yields were determined by high performance liquid chromatography. Modified two-step, multi-linear and non-linear regression analyses were conducted to determine the kinetic parameters of marker formation. Kinetic parameters were validated with experiments using a pilot-scale radio frequency sterilization system. M-1 formation followed a first order kinetics. The predicted M-1 yields based on the kinetics model agreed with experimental results. Accurate kinetic parameters for M-1 formation should enable us to use M-1 for developing and validating advanced thermal processes.

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Keywords: Kinetics; Chemical marker; Process validation; Dielectric heating; Radio frequency; Sterilization; Thermal processing

1. Introduction

Novel thermal processes such as dielectric heating can potentially improve the quality of shelf-stable sterilized food products. The most attractive feature of dielectric heating is its ability to volumetrically heat foods hermetically sealed in polymeric trays or pouches (Meredith, 1998). The volumetric heating allows rapid delivery of thermal energy to foods, thus minimizing overcooking commonly experienced in conventional thermal processing. Despite this attractive feature, microwave (MW) or radio frequency (RF) sterilization processes are not used in the US food industry. One important issue that needs to be addressed before the FDA approves dielectric sterilization is the development of a reliable procedure to evaluate and validate the required lethality for heat treatments to ensure microbiological safety.

To design a new thermal process that ensures adequate sterility for shelf-stable foods, it is necessary to determine the location of the cold-spot in packaged foods (FDA, 1977). Once the cold-spot is determined, accurate time–temperature data can then be gathered from the slowest heated point (Holdsworth, 1997) and used for developing appropriate thermal process procedures. Many temperature measurement devices such as thermocouples, infrared temperature sensors and fiber optic temperature sensors have drawbacks in dielectric heating studies (Berek, 1988; Goedeken, Tong, & Lentz, 1991). Thermocouples will alter electromagnetic fields in dielectric heating (Berek, 1988). In addition, large currents can be induced in the metal parts of the temperature sensors leading to false signals (Berek, 1988). Infrared cameras only measure surface temperature (Goedeken et al., 1991). Fiber optic temperature sensors, though not interfering with the electric magnetic field, provide measurements at only limited points in a food package (Berek, 1988).

In dielectric heating, heating patterns depend upon direct interaction between electromagnetic fields and
foods (DeCareau, 1985). Locations of the cold-spot within the foods during dielectric heating are not well defined. Thus, assessment of temperature distribution within hermetically sealed foods during dielectric heating is essential and data from only a small number of temperature measurements are inadequate. These problems prompted the development of time–temperature integrators as alternative process evaluation tools (Hendrickx et al., 1995; Kim, Taub, Choi, & Prakash, 1996).

Several biological integrators have been reported in the literature (Holdsworth, 1992; Mateu, Chinesta, Ocia, Garcia, & Martinez, 1997; Pflug, Jones, & Blanchett, 1980; Pflug, 1982; Rodriguez & Teixeira, 1988). Those integrators were used to indicate the relative safety of food products, but not for locating cold-spots. Microbiological assays are often time-consuming, expensive, subject to recovery and contamination problems, and require large population changes as evidence of the process (Holdsworth, 1997; Kim, Taub et al., 1996). An alternative approach is the use of thermostensitive chemical compounds to quantify the time–temperature history of food products. United States Army Natick Research Center developed effective chemical marker methods to assess heating uniformity in sterilized foods. Three markers were identified in various food systems, they are 2,3-dihydro-3,5-dihydroxy-6-methyl-(4H)-pyran-4-one (M-1), 4-hydroxy-5-methyl-3(2H)-furanone (M-2) and 5-hydroxymethylfurfural (M-3) (Kim & Taub, 1993). The marker yields can be correlated with the time–temperature effect within food systems, provided the kinetic information is obtained. Real foods in a chemical marker technique is the inconsistency of food composition leading to potentially large variations in the measurements of the marker yields. An alternate approach is to develop a consistent substrate for M-1 determination. In a previous study, we selected 20% whey protein gel as the substrate for the M-2 marker in microwave studies (Lau et al., 2003). This substrate can be immobilized as a bead system that can be embedded in foods to yield consistent results. For a given substrate, kinetic information on a marker yield can be helpful for prediction of the time–temperature effect in sterilized foods.

The objective of this study was to determine the rate constants and activation energy for the M-1 formation in whey protein gels at sterilization temperatures (116–131 °C). Three regression methods were used to estimate the Arrhenius parameters. The obtained parameters were then used to predict the M-1 yields in sterilization processes (radio frequency heating and retort treatment) and checked against the experimentally determined M-1 yield.

2. Materials and methods

2.1. Mechanism of the marker M-1 formation

Numerous compounds are thermally produced in foods through non-enzymatic browning and may be used as time–temperature integrators (Kato, Nakayama, Sugimoto, & Hayase, 1982). The ease of quantitative determination of the thermally produced compounds is a key requirement for chemical marker analyses. Kim and Taub (1993) selected three marker compounds as potentially useful markers, since they are formed from precursors readily available in foods and easily determined by HPLC. During a heating process, amino acids may interact with glucose through a non-enzymatic browning reaction to form Amadori compounds (Kato et al., 1982). Under a weak acidic condition (pH > 5),

### Nomenclature

- $A$: the molar concentration of glucose
- $B$: the molar concentration of amino acids
- $C_{100}$: cook value at 100 °C
- $E_a$: activation energy (kcal/mol)
- $F_0$: cumulative lethality (min)
- $M$: marker yield
- $t$: time (min)
- $k$: reaction rate constant
- $R$: the universal gas constant 1.987 cal/mol K
- $T$: temperature (K or °C)
- $m$: the reaction order to amino acids
- $n$: the reaction order to glucose
2,3-enolization leads to the formation of M-1 (2,3-dihydro-3,5-dihydroxy-6-methyl-4(h)-pyran-4-one) (Kim, Taub et al., 1996). A summary of reaction pathways leading to the M-1 is presented in Fig. 1.

The chemical reaction of M-1 formation can be represented as:

\[
\text{Glucose} + \text{R-NH}_2 \rightarrow \text{M-1}
\]

where \( A \), \( B \), and \( M \) are the molar concentrations of the reactants glucose, free amino groups in the whey protein chains, and product M-1. The function of the amino group is similar to that of a catalyst. The amino group was first combined with glucose in the Maillard reaction to form a number of intermediate compounds. However, at the advanced Maillard reaction stage, the amino group was liberated when the M-1 was formed (De Kok & Rosing, 1994). Therefore, as the reaction progressed, concentrations of glucose \( A \) and M-1 changed continuously with time, until the glucose was consumed. For the reaction expressed by Eq. (1), the reaction rate can be simply written as (Adamson, 1973):

\[
\frac{dM}{dt} = k \cdot A^n
\]  

where

\[
k = k_{\text{total}} B_m
\]

Eq. (2) suggests that reaction (1) becomes a pseudo nth order reaction. From previous published results (Kim & Taub, 1993) the formation of M-1 can be modeled by first order kinetics and, thus, Eq. (2) becomes:

\[
\frac{dM}{dt} = kA
\]

where

\[
A_0 + M_0 = A + M = M_\infty
\]  

and, therefore, Eq. (4) becomes:

\[
\frac{dM}{dt} = k(M_\infty - M)
\]  

Integrating Eq. (6) yields:

\[
-\ln(M_\infty - M) = kt - \ln(M_\infty - M_0)
\]

Rearranging Eq. (7):

\[
M = M_\infty - (M_\infty - M_0)e^{(-kt)}
\]

Values for \( M_0 \) and \( M_\infty \) were estimated by the non-linear fitting of Eq. (8) to experimental data at each temperature using a non-linear regression procedure of Sigma Plot (SPSS Inc., 1997).

2.2. Thermal treatments of whey protein gels in capillary tubes

To prepare whey protein gels, 20 g of whey protein concentrate (Alacen 882, containing 80% protein on dry basis, Santa Rosa, CA) and 2 g of glucose (Sigma, St. Louis, MO) were dispersed in 78 g of deionized water at 20 °C. Two milliliters of the dispersed solution were injected into the middle section of capillary tubes (1.5 mm inner dia.; 1.55 mm outer dia.; 150 mm in length). Both ends of the tube were sealed with a hot flame. During the sealing of the tubes, precautions were taken to avoid heating the whey solution. The whey solution in the sealed capillary tubes was heated for 40 min in a water bath set at 80 °C. The kinetic studies were carried out at 116, 121, 126 and 131 °C in oil baths with different time intervals to cover the possible temperature-time combinations used in radio frequency thermal treatment of low-acid foods (pH > 4.5) in large trays (25 min)(Wang, Wig, Tang, & Hallberg, 2003) or conventional thermal processes (up to 90 min). During the heating, care was taken to ensure that temperature was the only variable extrinsic factor, and intrinsic factors were kept constant. At the end of each heating time interval, the tubes containing whey protein gels were immediately removed from the heating bath and plunged into an ice water bath to rapidly cool the samples. Extraction of marker compound was carried out immediately after cooling. The experiments were carried out in duplicate.

2.3. HPLC analysis

An HPLC system was used to analyze M-1 yields in whey protein gels after the thermal treatments. The system consisted of a photodiode array detector (Hewlett-Packard 1040A, Plainsboro, NJ) and a solvent delivery system (ISCO model 2350, Lincoln, NE). The
system was controlled by a desktop computer and connected to an integrator. In preparing samples for HPLC analysis, heated whey protein gels were removed from capillary tubes and weighed. Then 0.2 g whey protein gels were homogenized in 4 ml of 10 mM H2SO4, centrifuged and filtered through 0.45 μm nylon membrane filters. The filtered solutions were injected into an HPLC Fact Acid Analysis Column (Bio-RAD, Hercules, CA) equipped with an automatic injection system (HP1050, Hewlett Packard Co., Plainsboro, NJ). The mobile phase was 10 mM H2SO4 at a flow rate of 1 ml/min at room temperature.

Absorbance of the effluent was determined at 295 nm as per Kim and Taub (1993). M-1 yields from the HPLC analysis were presented as the peak area observed divided by the total mass of the gel (Area/g).

3. Data analysis

According to Labuza and Baisier (1992), and Kim and Taub (1993), M-1 formation from the reaction of glucose and amino acids can be modeled as first order reactions. The kinetic parameters for M-1 formation were determined by modified two-step, multi-linear and non-linear regression methods based on a first order reaction. Discussion on the merits and disadvantages of each method for analyzing kinetics of chemical markers can be found in Lau et al. (2003).

3.1. Modified two-step regression

As mentioned in the mechanism of the marker formation, the formation of M-1 can be described by Eq. (8). The effect of temperature on the rate constants of marker formation was expressed as an Arrhenius relationship:

\[ k = k_{\text{ref}} e^{\left(\frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)\right)} \]  

where \( k_{\text{ref}} \) is the rate constant at the reference temperature \( T_{\text{ref}} \) (K), \( E_a \) is the activation energy and \( R \) is the universal gas constant (1.987 cal/mol K). The reference \( T_{\text{ref}} \) was taken as 123.5 °C or 396.8 K, the mean of the experimental temperature range. Eq. (9) was rewritten as:

\[ \ln k = \ln \frac{k_{\text{ref}}}{R} \left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right) \]  

With a normal two-step linear regression method, \( k \) values were estimated by first regressing the logarithmic reactant concentration versus time at each temperature, using Eq. (7). Arrhenius parameters (\( \ln k_{\text{ref}} \) and \( E_a \)) were then obtained by regressing \( \ln k \) versus \( 1/T \), using Eq. (10). In this study, the two-step method was slightly modified, because the value for \( M_\infty \) in Eq. (7) is needed prior to the determination of \( k \). The Marquardt–Levenberg algorithm for non-linear regression was first used to fit Eq. (8) to experimental data to obtain values for \( M_\infty \) and \( k \) at each temperature. Arrhenius parameters were then obtained by linear regression using Eq. (10).

3.2. 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 (Haralampu, Saguy, & Karel, 1985):

\[ -\ln(M_\infty - M) = \sum_{i=1}^{n} k_i T_i - \ln(M_\infty - M_0) \]  

where \( T_i \) is modified time variable. But we needed to have a prior knowledge of \( M_\infty \) before performing the multi-linear regression. The \( M_\infty \) value was first obtained from the non-linear regression method as described earlier, and used to calculate \( -\ln(M_\infty - M) \). A multi-linear regression was then conducted and \( k \) values at four different reaction temperatures were obtained by fitting the experimental data to Eq. (11). After obtaining \( k \) values at different reaction temperatures, Eq. (10) was used to determine the activation energy by linear regression.

3.3. Non-linear regression

Substituting Eq. (9) into Eq. (8), we obtain,

\[ M = M_\infty - (M_\infty - M_0) e^{\left(\frac{-E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)\right)} \]  

The non-linear regression procedures in Sigma Plot (SPSS Inc., 1997) were used to fit the marker yield \( \langle M \rangle \) versus time \( (t) \) and temperature \( (T) \) to Eq. (12) to obtain Arrhenius parameters \( (k_{\text{ref}} \) and \( E_a) \).

4. Results and discussion

4.1. Determination of \( M_\infty \) and \( M_0 \)

Table 1 shows the results of the non-linear analysis to determine \( M_0 \) and \( M_\infty \). An Analysis of Variance

<table>
<thead>
<tr>
<th>( T ) (°C)</th>
<th>( M_0 ) (peak area/g)</th>
<th>( M_\infty ) (peak area/g)</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>116</td>
<td>0.000 ± 0.091</td>
<td>1.667 ± 0.104</td>
<td>0.985</td>
</tr>
<tr>
<td>121</td>
<td>0.000 ± 0.072</td>
<td>1.750 ± 0.110</td>
<td>0.990</td>
</tr>
<tr>
<td>126</td>
<td>0.000 ± 0.089</td>
<td>1.758 ± 0.110</td>
<td>0.986</td>
</tr>
<tr>
<td>131</td>
<td>0.000 ± 0.075</td>
<td>1.854 ± 0.143</td>
<td>0.988</td>
</tr>
</tbody>
</table>
temperatures can be further answered by the literature and by our preliminary tests. Wolf, Thompson, and Reineccius (1978) monitored the change in lysine concentrations at elevated temperatures (80–130 °C) in Maillard reaction. After a loss of about 40–50% of available lysine the concentration of lysine remained constant even though glucose concentration continued to decrease with heating time. The fact that glucose concentration continued to decrease with time while lysine concentration remained unchanged can be explained by the Hodge-scheme where the amino acids/peptides were liberated and reused during the course of the advanced Maillard reaction phase (De Kok & Rosing, 1994). In our preliminary tests, M-1 yields were monitored as a function of heating time at 121 °C in 20% whey protein gels containing 2% or 5% glucose. The maximum M-1 marker yield $M_\infty$ was 2.5 times in whey protein gels containing 5% glucose as that in whey protein gels with 2% glucose. This finding further suggested that glucose was the limiting factor in the M-1 formation.

### 4.2. Rate constants and activation energy

The reaction rate constants and activation energy for M-1 formation were obtained by modified two-step, multi-linear and non-linear regression methods. The first-order reaction rate constants obtained from three regression analyses are summarized in Table 2. The rate constant increased with temperature. The $r^2$ value from modified two-step regression is smaller than those from multi-linear and non-linear methods. The modified two-step regression has the disadvantage of applying regression on regression coefficients, causing larger estimation errors (Cohen & Saguy, 1985; Haralampu et al., 1985; Van Loey, Fransis, Hendrickx, Maesmans, & Tobback, 1995), while the multi-linear regression analysis analyzed the data set as a whole. The multi-linear regression showed a better $r^2$ value than the modified two-step analysis, but the non-linear regression method resulted in the highest $r^2$ value among the three methods. Several researchers (Arabshahi & Lund, 1985; Cohen & Saguy, 1985) demonstrated that non-linear regression analysis provided the most accurate prediction of kinetic

![Fig. 2. M-1 formation in whey protein gels (2% glucose, 20% whey protein) when heated in oil baths as a function of time at different temperatures.](image)

Table 2

<table>
<thead>
<tr>
<th>Reaction rate Activation</th>
<th>Two-step regression</th>
<th>Multi-linear regression</th>
<th>Non-linear regression</th>
<th>Two-step regression*</th>
</tr>
</thead>
<tbody>
<tr>
<td>energy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{118}$ °C</td>
<td>0.0219 ± 0.0045</td>
<td>0.0149 ± 0.0075</td>
<td>0.0193 ± 0.0030</td>
<td>0.0099</td>
</tr>
<tr>
<td>$k_{121}$ °C</td>
<td>0.0277 ± 0.0056</td>
<td>0.0240 ± 0.0077</td>
<td>0.0288 ± 0.0034</td>
<td>0.0146</td>
</tr>
<tr>
<td>$k_{126}$ °C</td>
<td>0.0421 ± 0.0092</td>
<td>0.0351 ± 0.0179</td>
<td>0.0426 ± 0.0050</td>
<td>0.0213</td>
</tr>
<tr>
<td>$k_{131}$ °C</td>
<td>0.0567 ± 0.0127</td>
<td>0.0614 ± 0.0205</td>
<td>0.0625 ± 0.0096</td>
<td>0.0309</td>
</tr>
<tr>
<td>$k_{0f}$</td>
<td>0.0349 ± 0.0010</td>
<td>0.0299 ± 0.0009</td>
<td>0.0351 ± 0.0034</td>
<td>0.0198</td>
</tr>
<tr>
<td>$E_a$ (kcal /mol)</td>
<td>20.45 ± 1.6 ($r^2 = 0.989$)</td>
<td>28.92 ± 1.60 ($r^2 = 0.994$)</td>
<td>24.51 ± 2.43 ($r^2 = 0.995$)</td>
<td>23.7</td>
</tr>
</tbody>
</table>

*Kinetics information was obtained from broccoli extract (Kim & Taub, 1993).
parameters. Lau et al. (2003) also used the above three methods to develop a kinetic model for chemical marker M-2 formation in whey protein gels and found the non-linear regression method to be the most reliable one.

The \( E_a \) value obtained from the multi-linear regression analysis was the highest. The estimated Arrhenius parameters from multi-linear regression could be biased because the estimated reaction rates were not independent, thus violating the assumption for least squares regression (Haralampu et al., 1985). The estimated value for \( E_a \) using the non-linear regression yielded kinetic parameters very close to those reported by Kim and Taub (1993) for M-1 formation in broccoli extract (Table 2).

4.3. Validation of kinetic parameters under sterilization conditions

Since different kinetic parameters were obtained from three regression analyses, the question arose as to which values should be considered most accurate in the validation of thermal processing using the chemical marker method. A comparison was made between marker yield determined from whey protein gels from a radio frequency sterilization process and the marker yield predicted based upon measured temperature–time profiles and estimated kinetic parameters from the previous oil bath experiments. The sterilization experiments were conducted using 20% whey protein gels (292 × 229 × 49 mm) containing 2% glucose sealed in 6-lb capacity polymeric trays and processed in a steam retort and radio frequency (RF) system developed at Washington State University. A detailed description of the RF process can be found in Wang et al. (2003). Processing time and temperature at various locations of the whey protein gels were recorded using computer data acquisition systems. After heating, a small portion of the whey protein gel located near each temperature sensor was collected (5 mm diameter; 16 mm high) to determine M-1 yield.

The following equations were developed to predict the marker formation based on the determined \( E_a \) and \( k_{\text{ref}} \).

Substituting Eq. (9) into Eq. (6) yields:

\[
\frac{dM}{dt} = k_{\text{ref}} e^{\left(\frac{E_a}{R_1 T}\right)} (M_\infty - M) \tag{13}
\]

Integrating Eq. (13) gives:

\[
M(t) = M_\infty - (M_\infty - M_0) \times \exp \left[ \int_0^t k_{\text{ref}} e^{\left(\frac{E_a}{R_1 T}\right)} dt \right] \tag{14}
\]

The predicted M-1 yield was calculated by taking numerical integration of the right side of Eq. (14), using the measured time–temperature profile for whey protein gel thermally processed by RF and conventional retort methods (Fig. 3). The predicted M-1 yield based on the kinetic parameters obtained from the non-linear regression method agreed well with the experimental value (Table 3) except at the point in the corner of the tray for RF heating. The discrepancy might have been caused by slight differences in the locations where temperature was measured by fiber optic temperature sensor and where the sample was taken for chemical marker analyses. Specifically, the temperature was taken from a sensor located right next to the wall of the tray, and this sensor might respond more closely to changes in circulating temperatures.

![Fig. 3. Time–temperature profile for whey protein gel thermally processed by RF and conventional retort methods (Wang et al., 2003).](image-url)

<table>
<thead>
<tr>
<th>Processing method</th>
<th>Location</th>
<th>Calculated (peak area/g)</th>
<th>Measured (peak area/g)</th>
<th>( R_1 ) (min)</th>
<th>( C_{100} ) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF</td>
<td>Core</td>
<td>0.78</td>
<td>0.79</td>
<td>9.4</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>0.81</td>
<td>0.84</td>
<td>9.6</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>Corner</td>
<td>0.60</td>
<td>0.84</td>
<td>7.8</td>
<td>57</td>
</tr>
<tr>
<td>Retort</td>
<td>Core</td>
<td>1.15</td>
<td>1.18</td>
<td>10.6</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>1.19</td>
<td>1.12</td>
<td>12.0</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td>1.31</td>
<td>1.31</td>
<td>18.0</td>
<td>189</td>
</tr>
</tbody>
</table>

The calculated M-1 yields were based on parameters obtained from non-linear regression method (Wang et al., 2003).
water temperature (outside of the tray in RF heating, see Wang et al. (2003) for detailed description of the process) while the sample for chemical marker analyses was taken about 3 mm away from the corner. This might have resulted in a small calculated value for M-1 yield compared with the measured value.

Included also in Table 3 are estimated lethality \( F_0 \) and cook value \( C_{100} \) for different portions of the food processed with RF and retort systems. \( F_0 \) was calculated from (Stumbo, 1973):

\[
F_0 = \int_0^t 10^{\frac{t}{T}} \, dt
\]

where \( T \) is recorded temperature (°C), \( t \) is time (min), and \( z \) is a temperature-dependent factor in thermal inactivation kinetics, which is the temperature difference required to change the thermal death time by tenfold (°C) (Teixeira, 1992). A \( z \) value of 10 °C was used for *Clostridium botulinum* spores.

The Cook value, \( C_{100} \), was calculated from:

\[
C_{100} = \int_0^t 10^{\frac{t}{C_{100}}} \, dt
\]

A \( z \) value of 33 °C was used for describing the overall quality loss according to Lund (1986).

Values of \( F_0 \) and \( C_{100} \) and the corresponding marker M-1 yield for each of the three measured locations in 6-lb capacity polymeric trays after RF and conventional retorting processes are shown in Fig. 4. For the RF process, the M-1 yields were highly correlated to the values of \( F_0 \) and \( C_{100} \) \( (r^2 = 0.999 \text{ for both cases}) \). Similarly, for the retort process, \( r^2 \) was 0.998 for the correlation between M-1 and \( F_0 \), and 0.996 for the correlation between M-1 and \( C_{100} \). These high correlation coefficients suggest that chemical marker M-1 can indeed be an effective relative indicator not only for thermal lethality \( F_0 \) but also for cook value \( C_{100} \), when used to evaluate a novel thermal process, such as RF sterilization, or a conventional canning process. Within each process, M-1 yields can provide reliable information about the cold-spot in terms of thermal lethality as well as cooking uniformity.

M-1 yield may also be a very effective indicator of cook value when comparing the two different thermal processes, because the cook value increased almost linearly with M-1 yield over the whole range covered by RF and retort heating (Fig. 4). M-1 value is, however, not suited for comparing the thermal lethality of two totally different thermal processes (e.g., RF versus conventional retort). For example, for an \( F_0 \) of 10, the corresponding M-1 yield in the same tray was 1.1 for the retort heating and 0.9 for RF heating (Fig. 4). The above observations can be explained by the fact that the activation energy for M-1 formation in whey protein gels (24.5 kcal/mol by non-linear regression) is much closer to that for the cook value (19.7 kcal/mol, equivalent to \( z = 33 \) °C) than to that for thermal kill of *C. botulinum* (70.6 kcal/mol, equivalent to \( z = 10 \) °C). That is, the formation of chemical marker M-1 has a slightly higher but similar sensitivity to temperature change as that of cook value; M-1 yield can, therefore, be used to indicate cook value regardless of the thermal processes.

In summary, M-1 in whey protein gels with proper salt concentration can be used to simulate a selected food to determine the heating pattern in RF sterilization processes. Whey protein gels with glucose can also be embedded at various locations in the food to evaluate the time-temperature effect at each point in a real food system during thermal processes.

5. Conclusion

Reaction rates and activation energies of M-1 formation were obtained from the modified two-step, multi-linear and non-linear regression analyses. The predicted M-1 yield based on the kinetic parameters obtained from non-linear regression analysis agreed well with experimental values obtained from separate RF and retort sterilization tests. M-1 marker yields were highly correlated to thermal lethality value and cook value. This marker can be used to evaluate heating uniformity of a packaged food, either in a whey protein gel as a model food or in embedded whey protein gel beads.

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