

Kinetics of chemical marker M-2 formation in mashed potato—a tool to locate cold spots under microwave sterilization

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Received 27 November 2004; accepted 19 May 2005

Available online 9 August 2005

Abstract

Chemical marker M-2 (4-hydroxy-5-methyl-3(2H)-furanone) can be used as a tool to evaluate heating patterns of foods in microwave sterilization. This research studied the kinetics of the M-2 formation in mashed potato as influenced by temperature and salt content. Mashed potato (83.12% moisture content) with 1.5% D-ribose was heated in the capillary tubes at four temperature levels. Chemical marker M-2 yield was obtained using high performance liquid chromatography. Formation of M-2 in plain mashed potato was a first-order reaction. The rate constant changed with temperature following an Arrhenius relationship. For kinetic parameters estimation, one-step non-linear regression was the best followed by modified two-step regression. Amino acid was the limiting element in the formation M-2 in mashed potato. The salt content of 0–1% had no influence on the chemical marker yield. Addition of L-lysine more than 1% resulted in too dark color after sterilization treatments.

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Keywords: Chemical marker M-2; M-2 kinetics; D-ribose; L-lysine; Mashed potato; Microwave sterilization; Cold and hot spots

1. Introduction

Microwaves have been used widely in food processing operations, including drying, pasteurization and sterilization of foods. Because of the direct interaction between microwaves and food products, microwave volumetric heating can overcome the slow heat transfer between heating media and packaged foods during conventional heating (Ohlsson, 1992). A fast and reliable method to monitor and predict microwave-heating pattern in foods during sterilization is needed for successful development of commercial microwave sterilization processes. In order to design an effective thermal process to ensure adequate sterility for shelf-stable foods, it is

essential to determine the location of cold and hot-spots in packaged foods.

Microwave heating is different from conventional heating in which the heating patterns are dependent upon the direct interaction between microwave energy and food and are difficult to predict (Decareau, 1985). Thus, assessment of temperature distribution within packaged foods during microwave sterilization is essential, but it can not be determined with single point each or even various points temperature measurements (Ohlsson, 1972). Similar challenges were experienced in the development of 915 MHz single-mode microwave heating systems at Washington State University, USA. Issues that need to be addressed before obtaining regulatory and industrial acceptances include: determination of the locations of cold and hot spots and the nature of their mobility and repeatability, and development of reliable monitoring procedures to ensure a safe level of microwave sterilization (Guan, Plotka, Clark, & Tang, 2002). Kinetics of chemical marker M-2 formation in

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Nomenclature

C	chemical marker (M-2) concentration at any time (g/g of sample)	k_0	rate constant at reference temperature (min^{-1})
C_∞	chemical marker (M-2) concentration at saturation (g/g of sample)	n	order of the chemical reaction
E_a	activation energy for chemical reaction (kcal mol^{-1})	R_0	universal gas constant at reference temperature
F_0	cumulative thermal lethality (min)	t	time (min)
k	chemical reaction rate constant (min^{-1})	T	absolute temperature (K)

mashed potato has been studied (Kim, Taub, Choi, & Prakash, 1996a) to develop a method that can lead to the detection of cold and hot spot locations.

Direct measurement of time–temperature history for all points in food packages is not possible in microwave sterilization. Chemical marker offers an alternative as a time–temperature integrator to determine heating patterns. A chemical marker method was developed at the United States Army Natick Research Center (Kim & Taub, 1993) to determine heating patterns in food system for various thermal processes. Three markers 2,3-dihydro-3,5-dihydroxy-6-methyl-(4H)-pyran-4-one (referred to as M-1), 4-hydroxy-5-methyl-3(2H)-furanone (M-2) and 5-hydroxymethylfurfural (M-3) have been identified by scientists in the Natick US Army laboratory. Kim et al. (1996b) and Ramaswamy, Awuah, Kim, and Choi (1996) have used the M-1 yield as a temperature–time integrator to study ohmic heating and aseptic processing.

Chemical marker kinetics for M-1 and M-2 in whey protein gels have been studied (Lau et al., 2003; Wang, Lau, Tang, & Mao, 2004). In whey protein gel, reaction leading to M-2 formation was fast and ultimately giving a shorter time to reach the saturation point. M-1 cannot be used for high temperature short time processes. The Microwave Heating Group at Washington State University (Pullman, WA) selected mashed potato as a model food to locate cold and hot spots for approval of the sterilization system by regulatory bodies. Hence, kinetics information for M-2 in mashed potato was needed to monitor the microwave sterilization process qualitatively.

Understanding kinetics of the chemical marker M-2 formation in mashed potato as well as information about order of reaction, correlation of M-2 yield with cumulative lethality (F_0) will guide to develop a reliable method for determining heating patterns.

The objectives of this study were: (1) to determine the reaction order, rate constant and energy of activation for the M-2 formation in mashed potato at four temperature levels; (2) to find the limiting factor and the influence of different sources of mashed potato on the M-2 yield; (3) to study the M-2 formation over the range of

dielectric properties of model food by changing the salt content of mashed potato.

This kinetics study will help in establishing a process to develop a reliable method for determining the location of the cold and hot spots in 915 MHz microwave sterilization systems.

2. Materials and methods

Chemical marker M-2 (4-hydroxy-5-methyl-3(2H)-furanone) is formed by rearranging Amadori compound (Fig. 1) after the reaction between D-ribose and amino acids in the presence of weak acidic ($\text{pH} > 5$) environment (Prakash, Kim, & Taub, 1997). Early consumption of any component either D-ribose or amino acids will limit the yield of the chemical marker M-2 during sterilization processes. Amino acids, especially lysine, arginine, histidine and methionine, are of prime importance during formation of chemical marker M-2 in presence of D-ribose. A yield point after which there will be no significant effect of heating on chemical marker yield is called marker yield at saturation (C_∞). Chromatographic detection showed that chemical marker M-2 has a UV absorption maximum at 285 nm (Kim & Taub, 1993). Mashed potato used in this study contained added 1.5% D-ribose (Sigma, St. Louis, MO). Concentrations of amino acids in the sample were as follows: methionine 1.41 $\mu\text{M/g}$, lysine 1.7 $\mu\text{M/g}$, histidine 1.33 $\mu\text{M/g}$ and arginine 3.70 $\mu\text{M/g}$. Concentration of amino acids in plain mashed potato was much lower than that of D-ribose due to the chemical composition of potato. Equation for studying the kinetics of chemical marker (M-2) formation in mashed potato can be given as (Lau et al., 2003):

$$\frac{dC}{dt} = k(C_\infty - C)^n \quad (1)$$

The above equation conveys that the rate of formation $\frac{dC}{dt}$ is proportional to n th power of difference between concentration of marker yield at saturation, (C_∞ g/g of sample), and marker yield at any time, (C g/g of sample), while n is order of the reaction and k is reaction constant.

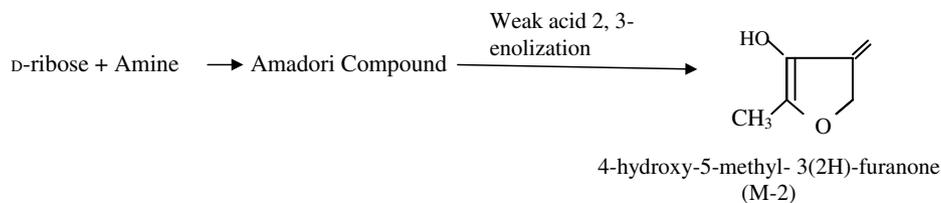


Fig. 1. Formation of chemical compound 4-hydroxy-5-methyl-3(2H)-furanone (M-2) in presence of D-ribose in mashed potato.

2.1. M-2 yield determination

Mashed potato was selected as a model food in the study because of its homogeneity and availability. During sample preparation, 1.5 g of D-ribose was dissolved first in 83.12 g of distilled water at room temperature and was then mixed with 15.38 g dry mashed potato flakes (Washington Potato Company, Warden, WA). About 2 ml of the dispersed paste was carefully injected into the glass capillary tubes (inner diameter = 1.75 mm; length = 15 cm). Both ends of the tubes were sealed with a hot flame. During tube sealing, precaution was taken to avoid heating of mashed potato. Experiments were carried using an oil bath set at 116, 121, 126 and 131 °C and at several time intervals in order to cover a range of likely temperature–time combinations in the microwave sterilization processes. At each temperature experiment was conducted in two replicates and both replicates were considered for estimation of kinetics parameters and order of reaction. The come-up time for the capillary tube, filled with mashed potato, to reach the set temperature level, was between 12 and 14 s. Tubes were heated in the oil bath at each temperature to upto 60 min. After predetermined heating times, the tubes were removed from the oil bath and immediately placed in a basin containing crushed ice. Cooled tubes were opened; the potato was removed and immediately weighed. A sample weighing between 0.16 and 0.17 g was placed in a mortar and carefully ground in 2 ml extraction buffer (10 mM sulphuric acid and 5 mM citric acid) according to the modified extraction procedure described by Lau et al. (2003). Extracts were collected and sealed tubes were stored overnight in a –20 °C freezer. Upon thawing at room temperature, extracts were mixed thoroughly and centrifuged for 10 min at 14,000 rpm (Eppendorf centrifuge, Brinkman Instruments, Westbury, NY). Supernatants were transferred into fresh tubes and were centrifuged again under the same conditions (10 min at 14,000 rpm). The supernatants were filtered using a PTFE syringe filter with a 0.45 µm pore size and placed into a high performance liquid chromatography sample holder for analysis to obtain M-2 yield.

The Agilent 1100 HPLC system (Agilent Technology, USA) was equipped with a diode array detector. Twenty five microliter aliquots were run through the fast acid

analysis column, 100 × 7.8 mm (Bio-Rad Laboratories, Hercules, CA). The mobile phase was 10 mM at a flow rate of 1 ml/min. Absorbance was determined at 285 nm as per Kim and Taub (1993). A calibration curve (Fig. 2) was established using a commercial sample of chemical marker M-2 obtained from Givaudan Flavor Corporation (Cincinnati, Ohio).

Standard dilutions were prepared using the same extraction buffer as for samples of mashed potato in kinetic studies. The calibration curve was used in expressing marker yield as mg of marker per gram of sample. The following equation was used in this study to express peak area of the sample as mg of marker per gram of sample:

$$\text{Marker yield} = \frac{\text{Peak area(sample)}}{57348} \times \frac{\text{Volume of extract}}{\text{Weight of sample}} \quad (2)$$

Initial M-2 yield in plain mashed potato could not be observed even at high sensitivity of the HPLC, indicating that the marker yield observed in the treated samples with ribose was indeed a product of reaction between added D(–) ribose and amino-acids endogenous to potato flakes during the heat treatments. The kinetic experiments were carried out in two replicates.

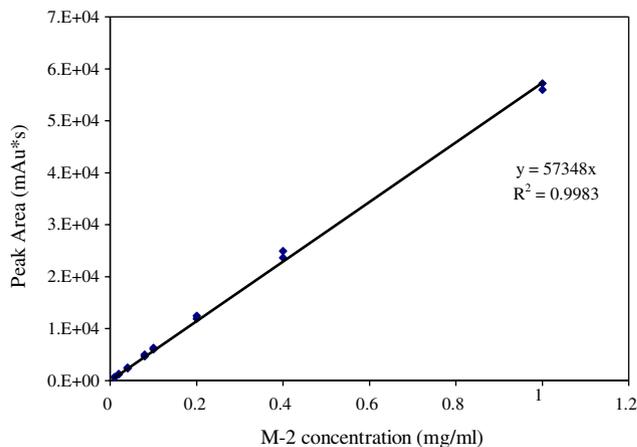


Fig. 2. Calibration curve of chemical marker M-2 (4-hydroxy-5-methyl-3(2H)-furanone) in 10 mM sulfuric-5 mM citric acid buffer.

2.2. Limiting factors M-2 formation

Instant mashed-potato flakes from two manufacturing lots: old (2002), new (2003) were acquired from Washington Potatoes Co. (Warden, WA) and were analyzed for amino acid composition. Since potato flakes are high in starch content (~66% according to the Data of National Grain and Feed Association), direct amino acid analysis with the raw material was not possible due to a high glucose concentration that interfered with the spectral detection of amino acids. Instead the potato flakes were pre-extracted prior to the amino acid analysis according to the modified method described by Yang (1997). One gram of flakes was extracted with 25 ml of 80% ethanol by shaking in a gyratory shaker (New Brunswick Scientific Precision Inc., M-3, Brunswick, NJ) at room temperature for 15 min and centrifuging at 1200g at 5 °C for 10 min. The supernatant was filtered through ashless Whatman filter paper of size 40 to remove potato particles. The total volume of extracts was brought up to 25 ml final volume with 80% ethanol. An aliquot was then applied to the column of Beckman 6300 Automatic amino acid analyzer (Beckman Instruments Co., Palo Alto, CA).

The moisture of potato flakes was determined according to the official AOAC method 7.003 (AOAC, 1980). Total protein content of potato flakes was determined using a Leco protein analyzer (Leco Corporation, St. Joseph, MI). All analyses of potato flakes samples were conducted in at least two replicates. Total protein content of mashed potato was 7.3% while the content of four major amino acids which participate in chemical marker M-2 formation were: methionine 1.41 µM/g, lysine 1.7 µM/g, histidine 1.33 µM/g, and arginine 3.70 µM/g.

To determine the limiting factor in M-2 formation, mashed potato sample was prepared with 1.5%, D-ribose. Chemical marker yield of the sample heated in an oil bath for predefined time at 121 °C temperature was obtained using HPLC. Experiments were repeated for higher levels of D-ribose: 3%, 4.5%. Levels of the D-ribose were selected to investigate the heating time at which amino acid content of the mashed potato become inadequate for M-2 formation. Chemical marker yield obtained at different levels were plotted to analyze the results. At each level five different time intervals were selected to obtain M-2 yield with two replicates.

2.3. Statistical analysis

Order of reaction and kinetics parameters can be determined by different statistic methods (Hill & Grieger-Block, 1980). Following three methods are commonly used: modified two-step, multi-linear regression and one-step non-linear regression analyses due to their ability to accurately estimate the kinetic parameters of

the Arrhenius model (Haralampu, Saguy, & Karel, 1985). It was reported however that among the above three methods, modified two-step regression gives least accurate estimation of the Arrhenius parameters because of the need to calculate many intermediate values before estimating the kinetics parameters (Haralampu et al., 1985). Multiple linear regressions showed bias with little improvement over modified two-step regression (Ramaswamy et al., 1996). Non-linear regression is probably the most appropriate theoretical method because it does not estimate unnecessary parameters (Arabshahi & Lund, 1984). These three methods were used to estimate the kinetics parameters in this study.

SAS Systems Release 8.1 (SAS Institute Inc., Cary, NC, 2000) was used to perform the statistical analysis. Integrating Eq. (1) between C_0 and C from time zero to time interval t we have:

$$\int_{C_0}^C \frac{dC}{(C_\infty - C)^n} = \int_0^t k dt \quad (3)$$

$$(C_\infty - C)^{1-n} - (C_\infty - C_0)^{1-n} = -(1-n)kt \quad (4)$$

$$(C_\infty - C) = [(C_\infty - C_0)^{1-n} - (1-n)kt]^{\frac{1}{1-n}} \quad (5)$$

The above equation does not apply to $n = 1$ because the term $\frac{1}{1-n}$ will become indeterminate. After rearranging the Eq. (5) we get:

$$C = C_\infty - [(C_\infty - C_0)^{1-n} - (1-n)kt]^{1/1-n} \quad (n \neq 1) \quad (6)$$

The above model was fitted with the experimental data using procedure NLIN (non-linear procedure) of SAS Systems Release 8.1 (SAS Institute Inc., Cary, NC, 2000). PROC NLIN fits non-linear regression models using the least square method. Experimental data supplied were marker yield at various time interval. The values estimated from the model were order of reaction, and marker yield at saturation (C_∞). Values of C_0 were considered as zero during estimation.

Coefficient of determination is the best measure to show the degree of fitting between experimental data and prescribed model. However, along with R^2 of non-linear regression we also validated the estimation using graphical analysis. In graphical analysis, exponent n in Eq. (1) was set to zero, half, one, and two to compare the coefficient of determination among zero-, half-, first-, and second-order reactions, respectively. Results obtained are presented in Table 2.

Fitting of the experimental data to model (6) predict the reaction to be of first order (Table 1). Graphical analysis also supports the predicted order of reaction. Estimation of the kinetic parameters was obtained considering $n = 1$ in Eq. (1), and integrating it between C_0 and C yields:

$$\ln(C_\infty - C) = \ln(C_\infty - C_0) - kt \quad (7)$$

Table 1
Estimated order of reaction (n), marker yield at saturation (C_∞) values by non-linear regression based on two replicates of experimental data

T (°C)	n	C_∞	R^2
116	1.050 ± 0.44	0.267 ± 0.04	0.989
121	1.0271 ± 0.23	0.284 ± 0.018	0.990
126	1.062 ± 0.18	0.273 ± 0.012	0.991
131	1.066 ± 0.40	0.268 ± 0.015	0.981

Eq. (6) in exponential form can be written as:

$$C = C_\infty - (C_\infty - C_0)e^{-kt} \quad (8)$$

The activated complex theory for chemical reaction rates is the basis for the Arrhenius equation, which relates reaction rate constants to the absolute temperature. The Arrhenius equation is (Holdsworth, 2000):

$$k = Ae^{-\frac{E_a}{RT}} \quad (9)$$

where E_a is the activation energy (kcal/mol), A the rate constants, T the absolute temperature (K) and R the universal gas constant (1.987 cal/mol K). Another form of the Arrhenius equation involves the reaction rate constant at a reference temperature. Under this study reference temperature T_0 (396.7 K) was taken as average of all four temperature considered. If k_0 is the reaction rate constant at T_0 then Eq. (9) can be modified as:

$$k = k_0 e^{-\frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_0} \right)} \quad (10)$$

Eq. (10) can be written in linear form by taking logarithmic on the both sides:

$$\ln k = \ln k_0 - \frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_0} \right) \quad (11)$$

Following three statistical methods: modified two-step, multi-linear and one-step non-linear regression methods were considered for estimation of the kinetics parameters.

(i) *Method I*: In *Modified two-step* regression method experimental data were fitted to the model (8) using NLIN procedure of SAS, which is based on the Marquardt algorithm to obtain C_∞ and k at each temperature. Marquardt iterative algorithm regresses the residuals onto the partial derivatives of the model with respect to the parameters until the estimates converge. Reaction

rate constants (k) at all temperatures were fitted into the linear model (11) to obtain $\frac{E_a}{R}$ as slope and $\ln(k_0)$ with intercept term. Energy of activation E_a and k_0 were calculated from the slop and intercept term, respectively.

(ii) *Method II*: In *Two-step multi-linear regression*, times were introduced as pseudo-dummy variables t_{Ti} at different temperatures, T_i in Eq. (7). The dummy time variable was created by associating the reaction times at a particular temperature, T_i , with a parameter, k_i , and setting the dummy times associated with the other temperature levels to zero. In this study, m was taken as four because of four temperature levels:

$$-\ln(C_\infty - C) = \sum_{i=1}^m k_i t_{Ti} - \ln(C_\infty - C_0) \quad (12)$$

Detailed information on using Eq. (12) is provided in Haralampu et al. (1985). C_∞ value obtained from non-linear regression method was used in calculating $\ln(C_\infty - C)$. $\ln(C_\infty - C)$ and pseudo-dummy variables t_{Ti} were fitted with model (12) to obtain reaction rate constant k_i at each temperature level. After all the k values at different reaction temperatures were obtained, they were fitted to model (11) and E_a , k_0 were estimated same as in case of modified two-step regression.

(iii) *Method III*: One-step non-linear regression performs a single regression on all of the data to estimate $\frac{E_a}{R}$ and k_0 without calculating the reaction rate at each temperature. An equation without reaction rate constant can be obtained by replacing the k terms in Eq. (8):

$$C = C_\infty - (C_\infty - C_0) \times \exp \left\{ -tk_0 e^{-\frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_0} \right)} \right\} \quad (13)$$

Substituting k in Eq. (7) with Eq. (8), we obtain:

$$C = C_\infty - (C_\infty - C_0) \times \exp \left\{ -tk_0 \exp \left[-\frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_0} \right) \right] \right\} \quad (14)$$

Transformation of the equation to this form improves the accuracy of the estimation (Nelson, 1983). The non-linear regression procedure in SAS Systems was used to fit the marker yield (C) versus time (t) data to Eq. (14), to estimate the E_a and K_0 at each temperature level. Reaction rate constant at each temperature was calculated back using Eq. (11).

Table 2

Estimation of the order of chemical marker (M-2) formation by examining r^2 from plot of zero-, half- and second-order reactions based on two replicates of experimental data

Temperature (°C)	Zero-order ($C_\infty - C$) time	Half-order ($C_\infty - C$) ^{0.5} versus time	First-order $\ln(C_\infty - C)$ versus time	Second-order $1/(C_\infty - C)$ versus time
116	0.855	0.921	0.987	0.715
121	0.866	0.942	0.973	0.765
126	0.829	0.917	0.978	0.776
131	0.672	0.672	0.959	0.416

2.4. Effect of salt content and additional L-lysine on M-2 yield

Mashed potato with 0% or 1% salt content was filled into the capillary glass tubes and experiment was carried out at 121 °C. Tubes were taken out after 5 min intervals from an oil bath and samples were prepared for HPLC analysis as mentioned above. M-2 yield obtained after HPLC analysis at each salt level with two replicates were plotted to determine the effect of salt level on M-2 yield.

Effects of additional L-lysine on chemical marker yield was studied with added L-lysine, ranging from 0.5%, 1.0%, and 2.0%. Mashed potato sample containing L-lysine in specified proportions were filled into tubes and heated at 121 °C. Samples containing 2% L-lysine taken out at 5 min interval was very dark because the marker yield reached the saturation point. Microwave sterilization processing lower than 5 min is not anticipated on industrial scale systems. Hence, we discarded any experiment with additional L-lysine higher than 2%. Chemical marker yield of Potato Buds (8.7% protein), Russet potato (5.2% protein) and Idahoan Real potato (8.69% protein) having different brands and ingredients were also tested to compare with old and new batch of mashed potato.

3. Results and discussion

Increase in marker yield (M-2) as a function of time at four different temperatures are shown in Fig. 3. Those data were used to determine kinetics information for

formation of M-2 in mashed potato. Chemical marker yield (M-2) in the unheated mashed potato sample was considered as zero, because no peak was detected during HPLC analysis. An analysis of variance (ANOVA) shows that the marker yields at saturation level for different temperatures were not different on 95% confidence interval. Estimated marker yield at the saturation level matched with the marker yield obtained by HPLC analysis.

3.1. Estimated chemical reaction parameters

Statistic analysis indicated that the chemical marker formation in mashed potato followed a first-order reaction. Graphical analysis also confirmed the first-order of reaction. Lau et al. (2003) and Wang et al. (2004) obtained a first-order reaction for M-2 and M-1 formation in whey protein gels. Table 1 summarizes the order of reaction and chemical marker yield at saturation. Results obtained by the graphical analysis for reaction order are presented in Table 2.

First-order kinetics ($n = 1$) for M-2 formation was used to calculate the reaction constant and activation energy. Kinetic parameters (k, E_a) for M-2 formation were obtained by modified-two-step, two-step multi-linear and one-step non-linear regression methods. Results obtained using these statistical methods are given in Table 3. The standard error in reaction rate constant for one-step non-linear regression was calculated using:

$$\Delta k = k \left[\left| \frac{\Delta k_0}{k_0} \right| + \left| \frac{\Delta E_a}{R} \left(\frac{1}{T} - \frac{1}{T_0} \right) \right| \right] \quad (15)$$

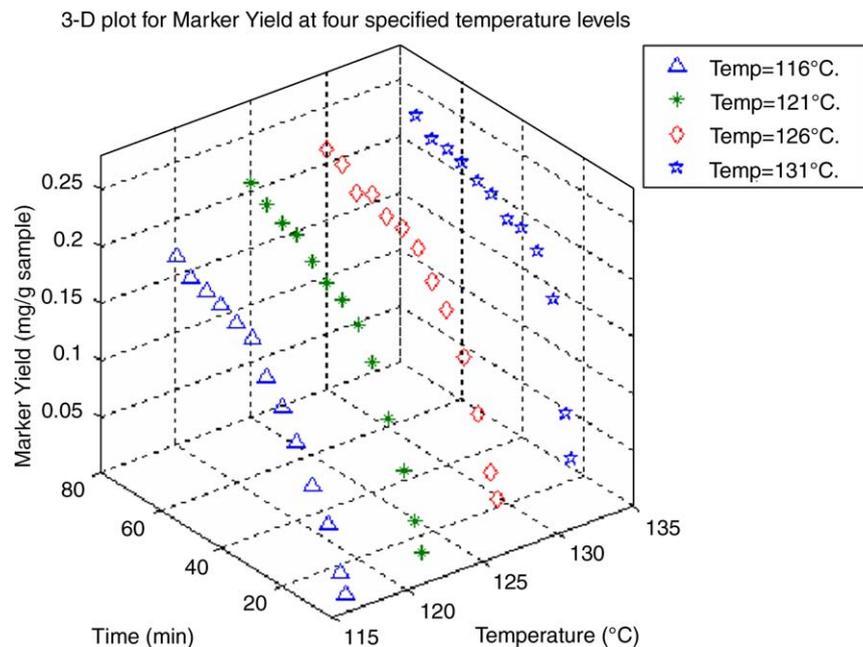


Fig. 3. Chemical marker yield (M-2) for different temperature levels obtained during experimental work, scattered data represent means of two replicates.

Table 3

Rate constant (min^{-1}), and activation energy, E_a (kcal/mol), for M-2 formation in mashed potato at four temperatures levels based on two replicates of experimental data

Reaction rate, activation energy	Modified two-step regression	Two-step multi-linear	One-step non-linear regression	M-2 formation with whey protein gel (Lau et al., 2003)
k_{116}	0.031 ± 0.014	0.029 ± 0.004	0.030 ± 0.001	0.110
k_{121}	0.040 ± 0.007	0.0451 ± 0.004	0.044 ± 0.002	0.152
k_{126}	0.056 ± 0.007	0.053 ± 0.005	0.063 ± 0.003	0.207
k_{131}	0.096 ± 0.024	0.072 ± 0.006	0.089 ± 0.005	0.281
k_0	0.050 ± 0.048	0.048 ± 0.066	0.052 ± 0.002	0.178
E_a (kcal mol $^{-1}$ K $^{-1}$)	22.96 ± 2.73 $r^2 = 0.978$	19.45 ± 3.79 $r^2 = 0.914$	22.23 ± 1.54 $r^2 = 0.983$	19.48

Coefficient of determination (r^2) and standard error for k , and E_a estimated are provided in Table 3. One-step non-linear method is the best among considered methods followed by the modified two-step method based on the values of r^2 and the standard error. One step non-linear method has advantage of using whole data set, as well as estimating fewer parameters during analysis. The multi-linear regression gives the smallest r^2 and the largest standard error for estimation of activation energy.

Activation energy estimated for M-2 formation (22.23 ± 1.54 kcal/mol) is within the range cited in the literature for non-enzymatic browning on in fruits and vegetables (ranging from 16 to 30 kcal/mol) (Labuza & Baisier, 1992). Kinetic parameters estimated in this study were compared with the kinetic parameters for M-2 formation with whey protein gel (Lau et al., 2003) in Table 3. Reaction rate constant (k) increases with temperature for both cases. Mashed potato has lower amino acid content than whey protein gel, which resulted in lower k values.

3.2. Estimation of limiting factor

Chemical marker yield increased with time over 60 min for 1.5% and 3% D-ribose while yield reached to the saturation point around 20 min with 4.5% D-ribose as shown in Fig. 4. This suggests that the amino acid content in mashed potato was adequate for 1.5%, 3% D-ribose until 60 min, while amino acid was consumed completely around 20 min in case of 4.5% D-ribose (Fig. 4). Analytical study showed that total amino acid content in 15 g of mashed potato is much less compare to 1.5 g D-ribose. Hence, acid content was observed as the limiting factor during chemical marker M-2 formation.

3.3. Estimation of effect of additional lysine

L-lysine content higher than 2% was discarded due to fast reaction and shorter time to reach saturation. L-lysine above 0.5% produced dark color and reduced the saturation time to less than 5 min at 121 °C. Mashed

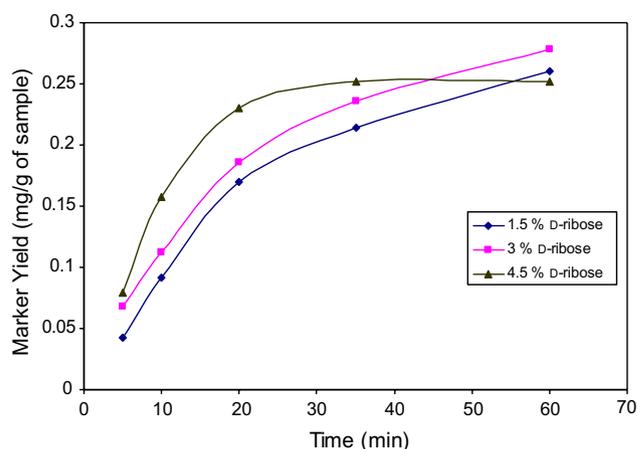


Fig. 4. Chemical marker (M-2) yield with different percentage of D-ribose at 121 °C, each point represent mean of two replicates.

potato with 0.5% L-lysine had higher yield than plain mashed potato. This study showed that quick appearance of saturation especially at hot spots would be misleading. A longer linear range for marker increase with time was observed with plain mashed potato in comparison of 0.5% L-lysine (Fig. 5). Moreover, L-lysine is costly so that determination of hot and cold spots for microwave sterilized foods was recommended without L-lysine.

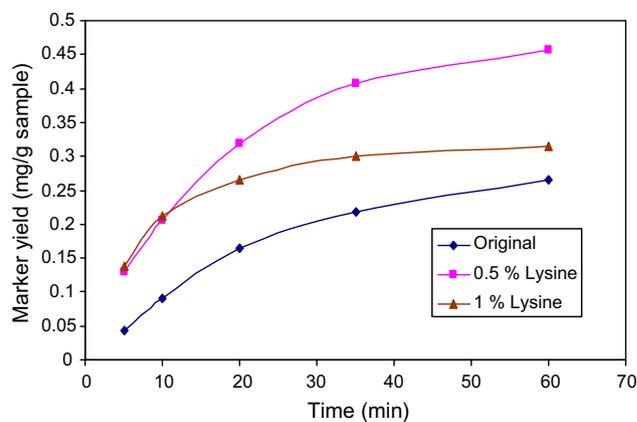


Fig. 5. Effect of additional L-lysine on marker yield in plain mashed potato at 121 °C, each point represents the mean of two replicates.

3.4. Variability among different mashed potato sources

Three locally available brands of potato flakes: Potato Buds (8.7% protein), Russet potato (5.2% protein) and Idahoan Real potato (8.69% protein) along with old and new batches of mashed potato flakes (8.3% and 7.3% protein, respectively) were tested for estimating the variability of mashed potatoes sources on chemical marker yield. M-2 yield obtained (Fig. 6) were statistically analyzed using SAS software. M-2 yields from different sources of potato were not significantly different (P -value = 0.989).

3.5. Effect of salt on marker yield

Two replicates for each level of salt were evaluated and statistical analysis of chemical marker yield was performed using SAS software. Analysis ($P > 0.9988$) showed that chemical marker yield was independent of the levels of salt content (Fig. 7).

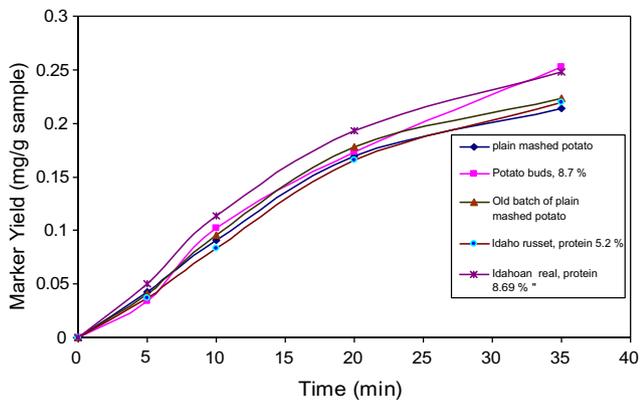


Fig. 6. Chemical marker (M-2) yield for various sources of mashed potato at 121 °C, each point represents the mean of two replicates.

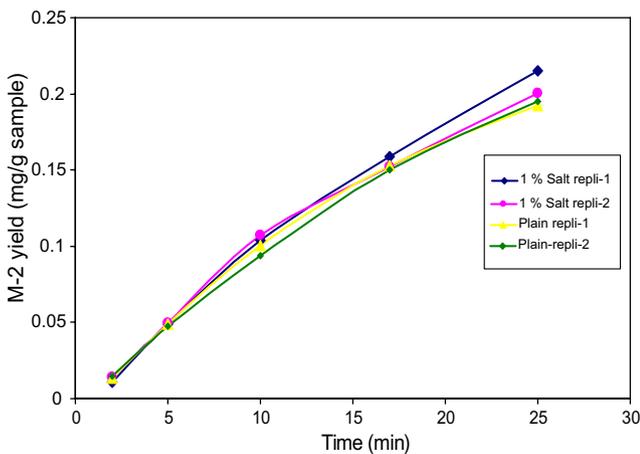


Fig. 7. Effect of salt content on chemical marker (M-2) yield at 121 °C, each level has two replicates.

4. Conclusion

Chemical marker (M-2) formation in mashed potato with 1.5% D-ribose was predicted as a first-order reaction. Kinetic parameters were predicted much accurately by one-step non-linear regression method followed by modified two-step regression. One-step non-linear regression was a more appropriate method since it does not estimate unnecessary parameters and gives both unbiased and precise estimation of the parameters. Modified two-step regression was a second option with higher standard error in estimation of the activation energy. Multiple linear regression method gives broader confidence interval in the estimation of activation energy along with the lowest overall coefficient of determination (r^2). Amino acid content was the limiting factor in formation of M-2 from D-ribose and mashed potato. Chemical marker yields of the considered sources of potatoes were not significantly different. Marker yield was found to be independent of the level of salt. The kinetic parameters obtained in this study can be used for determining the hot and cold spots locations during high temperature short time microwave sterilization processes.

Acknowledgements

This research work was supported by the US Army Natick Soldier Center, Natick, MA, and WSU microwave sterilization consortium that consists of Kraft Foods, IL, Ferrite Components, NH, Hormel Foods, MN, MasterFoods, CA, Ocean Beauty SeaFoods, WA, Graphics Packaging, CO, Rexam Containers, MO, and Washington State University, WA.

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