



ANALYSIS OF THERMOXIDATION KINETICS OF MILK FAT

ANÁLISIS DE LAS CINÉTICAS DE TERMOXIDACIÓN DE GRASA BOVINA

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Abstract

The thermoxidation of four different milk fat samples was studied using isoconversional method. The milk samples contained different ratio of unsaturated to saturated fatty acids of 0.7, 0.86, and 1.36 (MF1, MF2, and MF3, respectively), and a hydrolyzed milk fat sample (90% free fatty acids) with a ratio of 1.40 (MF4). The thermoxidation has been conducted under non-isothermal oxidation from 380 to 520K using five different heating rates (3, 6, 9, 12 and 15 K min⁻¹). The Kissinger-Akahira-Sunose (KAS) method was used to calculate the activation energy as a function of the extend of themroxidation. The obtained dependencies were interpreted in terms of oxidation mechanisms. The activation energy values were 100.10, 84.53, 80.41, and 121.44 kJ mol⁻¹ for MF1, MF2, MF3, and MF4, respectively. The thermoxidaiton of milk fat in the form of triacylglycerol was best described by Avrami-Erofeev model (A3), while the thermoxidation of free fatty acids was described by a frist-order model (F1). The calculated kinetic triplet were used to simulate the thermoxidation of milk fat and showed good agreement with the experimental data.

Keywords: non-isothermal oxidation, bioactive lipids, kinetic parameters, isoconversional method.

Resumen

Se estudió la termoxidación de la grasa bovina en cuatro diferentes muestras utilizando el método de isoconversión. Las muestras de grasa bovina contenían una proporción de ácidos grasos saturados con respecto a ácidos grasos no saturados de 0.7, 0.86, y 1.36 (MF1, MF2, y MF4, respectivamente), y una muestra hidrolizada con una proporción de 1.40 (MF4). La termoxidación fue realizada bajo condiciones no isotérmicas en un intervalo de temperatura de 380 a 500 K utilizando cinco diferentes velocidades de oxidación (3, 6, 9, 12, y 15 K min⁻¹). Se utilizó el método de Kissinger-Akahira-Sunose (KAS) para estimar las variaciones de la energía de activación con respecto al grado de oxidación. Tales dependencias fueron interpretadas en base a mecanismos de oxidación. Los valores obtenidos de la energía de activación fueron 100.10, 84.53, 80.41, and 121.44 kJ mol⁻¹ para MF1, MF2, MF3, y MF4, respectivamente. La termoxidación de grasa bovina en forma de triglicéridos fue representada por un modelo de Avrami-Erofeev (A3), mientras que la termoxidación de los acidos grasos libres fue satisfactoriamente representado por medio de un modelo de primer orden (F1). Se utilizaron los parámetros cinéticos para simular la termoxidación de la grasa bovina y se observó que los parámetros calculados pueden satisfactoriamente representar los datos experimentales.

Palabras clave: oxidación no isotérmica, lípidos bioactivos, parámetros cinéticos, métodos de isoconversión.

1 Introduction

Milk fat is an essential ingredient for the food and dairy industry due to nutritional, functional, and sensory contributions. Over the last few years, scientific evidence has shown that the consumption of some minor fatty acids naturally occurred in milk can lower the risk of certain diseases. These fatty acids are known as bioactive or healthy lipids, and they provide additional health benefits beyond

their basic nutrition (Shingfield *et al.*, 2008; Wang *et al.*, 2012). Examples of healthy lipids found in milk are conjugated linoleic acid (CLA), *trans*-vaccenic acid (TVA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and phospholipids. The potential health claims associated with the consumption of bioactive milk lipids have been extensively reviewed (Molkentin, 2000; McCrorie *et al.*, 2011; Cilla *et al.*, 2016).

Bioactive lipids in milk are present in relatively low concentration (between 0.5 to 2% of the total milk fat), which limits the use of milk for delivering

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bioactive lipids in the human diet (Martinez-Monteagudo and Saldana 2014, 2015). Strategies to further utilize bioactive lipids in milk have become a topic of research and commercial interest (Rodríguez-Alcalá *et al.*, 2013; Martinez-Monteagudo *et al.*, 2015). One approach for increasing the concentration of bioactive lipids in milk consists in developing nutritional strategies for dairy cattle that influence the milk fat composition (Shingfield *et al.*, 2008). The majority of studies involving modifications of the fatty acid profile in milk have evaluated the effect of forage species, oils and oilseeds, fish oil and marine algae supplements (Ryhänen *et al.*, 2005; Zheng *et al.*, 2005; Bell *et al.*, 2006; Bharathan *et al.*, 2008).

Increasing the concentration of bioactive lipids in milk fat through nutritional manipulation of dairy cattle has created new opportunities for development of milk fat based ingredient and products (Jenkins and McGuire 2006). Interestingly, nutritional protocols aimed to increase the concentration of a target group of fatty acids result in higher proportion of unsaturated fatty acids with respect to saturated fatty acids. This is an important consideration from a processing standpoint because unsaturated fatty acids are more prone to oxidation than saturated fatty acids. Indeed, some authors reported significant losses of CLA during thermal processing of enriched and fortified milk (Campbell *et al.*, 2003; Herzallah *et al.*, 2005; Martinez-Monteagudo and Saldana 2015).

Oxidation of milk fat leads to the development of unpleasant flavor and aroma, loss of nutrients, and formation of toxic compounds. The oxidation of milk fat is monitored according to the formation of primary (hydroperoxides, peroxide value, etc.,) and secondary oxidation products (TBARS, hexanal, octanal, etc.,). However, in the case of bioactive lipids (CLA, TVA, EPA, and DHA), such determinations might not represent the actual oxidation level. For instance, Hamalainen *et al.* (2001) found that conjugated fatty acids have more than one type of primary oxidation products, including monomeric and cyclic peroxides. Similarly, Yurawecz *et al.* (2003) oxidized methyl esters of CLA and found that the majority of oxidation products were dimers and polymers rather products of hydroperoxides breakdown. Thus, measuring oxidation indicators for bioactive milk lipids requires advanced analytical methods.

Alternately, differential scanning calorimetry (DSC) has been used to rank and classify lipids in terms of their oxidative stability. A common feature of DSC for measuring oxidation is the difficulty in selecting a kinetic model that enables

the evaluation of the kinetic triplet, pre-exponential factor, activation energy, and reaction model (A , E_a , and $f(\alpha)$), respectively. Isoconversional methods have the ability to calculate the kinetic parameters regardless of the reaction mechanism. This is because isoconversional methods describe the kinetics of the process by using multiple single-step kinetic equations, each of which is associated with some degree of conversion (Vyazovkin and Sbirrazzuoli 2006). Fundamentals and applications of isoconversional kinetic models are provided elsewhere (Vyazovkin and Wight 1997; Šimon 2004; Vyazovkin and Sbirrazzuoli 2006; Vyazovkin *et al.*, 2011). Martinez-Monteagudo *et al.* (2011) used isoconversional kinetics to evaluate the non-isothermal oxidation kinetics of anhydrous milk fat samples containing different concentration of unsaturated fatty acids. It was found that the start temperature of oxidation obtained from DSC analysis can effectively differentiate the oxidative stability between samples. The current investigation is aimed at providing a kinetic analysis of the thermoxidative behavior of milk fat containing different concentration of bioactive lipids. Such analysis includes computation of pre-exponential factor and activation energy, variations of activation energy during non-isothermal oxidation, and identification of suitable reaction model.

2 Materials and methods

2.1 Milk fat samples

The milk fat samples were obtained from the Dairy Research and Technology Centre, University of Alberta, Canada. Briefly, three different feeding regimes supplemented with safflower oil and vitamin E were provided to dairy cattle for 21 d following the methodology described elsewhere (Bell *et al.*, 2006). Then, the raw milk was warmed and centrifuged at 6000 x g for 6 min using an Alpha-Laval centrifuge (LAPX 202, Lundm Scania, Sweden). The cream obtained was stirred for 20 min at room temperature using a hand Black Decker Power Pro Mixer. The butter milk was discarded and the butter was washed with cold water to remove excess of butter oil. The butter was heated at 335 K for 120 min until the different layers started to separate. Then, the top layer was removed and the mixture was poured through cheese cloth to obtain milk fat (99.8%). This fat was stored at -18°C until further analysis.

2.2 Non-isothermal oxidation data

The DSC oxidation spectra (heat flow vs temperature) obtained by Martinez-Monteagudo *et al.* (2011, 2014) were used for the kinetic analysis. The oxidation kinetics were performed in a Q100 Modulated Differential Scanning Calorimeter (TA instruments, New Castle, DE). Samples of 1-2 mg were placed into aluminum pans with a pinhole lid (TA instruments, New Castle, DE) and were hermetically sealed. An empty sealed aluminum pan was used as a reference and the experiments were performed under an oxygen (dry 99% pure, Praxair) flow rate of 50 mL/min at 20 psi. These conditions allowed the interaction of the sample and the oxygen and were kept constant during the entire heating protocol. The sealed pans were equilibrated at 375 K for 1 min and then heated to 575 K at linear increased program rates (3, 6, 9, 12, and 15 K min⁻¹) to generate the oxidative profile (heat flow against temperature). These spectra include three milk fat samples containing a ratio of unsaturated to saturated fatty acids of 0.7, 0.86, and 1.36 (MF1, MF2, and MF3, respectively), and a hydrolyzed milk fat sample (90% free fatty acids) with a ratio of 1.40 (MF4).

2.3 Degree of conversion

The spectra were analyzed with a TA Universal Analysis software (TA instruments, New Castle, DE) to locate the start temperature (T_s) and maximum heat flow temperature (T_p) using the first and second derivatives of the signal as described elsewhere (Martinez-Monteagudo *et al.*, 2011; Saldaña and Martínez-Monteagudo 2013). The corresponding heat flow at the start temperature of oxidation was used as initial value of the heat flow (HF_o), while the corresponding heat flow at the maximum heat flow temperature was considered as the final heat flow (HF_f). Then, the degree of conversion (α) was obtained using the heat flow signal at a given temperature (HF_T) as a function of temperature, according to Equation (1):

$$\alpha = \frac{HF_o - HF_T}{HF_o - HF_f} \quad (1)$$

Equation (1) assumes that oxidation is completed when the temperature of maximum heat flow is observed in the DSC spectra. A degree of conversion equal to 1.0 corresponds to the maximum heat flow temperature, which in turns relates to the termination stage of oxidation. This relation (α and T_p) has

been validated by measuring the concentration of hydroperoxides for numerous edible oils (Ulkowski *et al.*, 2005).

2.4 Influence of degree of conversion on activation energy

The dependency of activation energy on the degree of conversion was evaluated using the Kissinger-Akahira-Sunose (KAS) method. This method is based on the principle of using multiple single-step kinetic equations to describe a given kinetic process. Each equation is associated with a certain extend of conversion and a narrow range of temperature related to that such conversion. The influence of the temperature on the kinetic process follows the Arrhenius law. Equation (2) illustrates a generic form of isoconversional kinetics.

$$\frac{d\alpha}{dT} = \frac{A}{\beta} \exp\left(\frac{-E}{RT}\right) f(\alpha) \quad (2)$$

The KAS method is an integral isoconversional method consisting in computing the activation energy at selected regular α increment covering the range of 0.05 to 0.95. Since the integral of Equation (2) does not have an analytical solution, the KAS method uses an approximation of the integral in the form of Equation (3), where the activation energy at a constant degree of conversion (E_α) is obtained by plotting $\ln(\beta_i/(T_{\alpha,i}^2))$ against $1/T_{\alpha,i}$.

$$\ln\left(\frac{\beta_i}{T_{\alpha,i}^2}\right) = Const - \frac{E_{\alpha,i}}{RT_{\alpha,i}} \quad (3)$$

Where β_i is the constant heating rate; T_α is the temperature at a constant α ; R is the universal gas constant.

2.5 Reaction model

A kinetic analysis of non-isothermal thermoxidation data for constant heating rate follows the form of Equation (4), where the influence of temperature is represented by the rate constant, $k(T)$, while the influence on the degree of conversion is represented by the reaction model, $f(\alpha)$.

$$\beta\left(\frac{d\alpha}{dT}\right) = k(T)f(\alpha) \quad (4)$$

The rate constant follows the Arrhenius law, according to Equation (5):

$$k(T) = A \exp\left(\frac{-E_a}{RT}\right) \quad (5)$$

where A is the pre-exponential factor; E_a is the overall activation energy. After some arrangements, Equation (4) can be expressed as follows:

$$\frac{d\alpha}{f(\alpha)} = \frac{A}{\beta} \exp\left(\frac{-E_a}{RT}\right) dT \quad (6)$$

Upon integration of Equation (6) leads to:

$$\int_0^\alpha \frac{d\alpha}{f(\alpha)} = \frac{A}{\beta} \int_0^T \exp\left(\frac{-E_a}{RT}\right) dT \quad (7)$$

The left side of Equation (7) is the integral form of the reaction model, $g(\alpha)$. The integral of the right side of Equation (7) does not have an analytical solution. In this work, the integral was solve using the fourth-degree solution of Senun and Yang approximation (Pérez-Maqueda and Criado 2000), Equation (8).

$$g(\alpha) = \frac{AE_a}{\beta R} \exp^{(-x)} \left[\frac{\pi(x)}{x} \right] \quad (8)$$

$$\pi(x) = \frac{x^3 + 18x^2 + 88x + 96}{x^4 + 20x^3 + 120x^2 + 240x + 120}$$

Where x is the dimensionless activation energy, $E_a/(RT)$. Table 1 shows the seven different reaction models used in this study.

2.6 Computation of pre-exponential factor

Each reaction model yields a set of kinetic triplets (A , E_a , and $g(\alpha)$). Out of these set of parameters, A_i and E_i varies linearly with the heating rate, according to Equation (9). From this relation, a and b are calculated and further substituted in Equation (10) to calculate A_o , using the value of activation energy that correspond to E_α .

$$\ln A_i = aE_i + b \quad (9)$$

$$\ln A_o = aE_o + b \quad (10)$$

2.7 Discrimination of the reaction models

The calculations were done using the software package AthenaVisual Studio, which supplies posterior probabilities as well as multivariate goodness of fit criteria. The different tested models were discriminated based on the coefficient of determination (R^2), the adjusted coefficient of determination (R_{Adj}^2), residual analysis, and residual sum of squares (RRS).

$$RSS = \sum (y_{exp} - y_{cal})^2 = \min \quad (11)$$

Where y_{exp} and y_{cal} are the experimental and calculated degree of conversion, respectively.

3 Results and discussion

3.1 Degree of conversion

The degree of conversion (thermoxidized fraction) of milk fat is shown in Figure 1. In general, the kinetic curves for all samples were sigmoidal in shape resembling the behavior of an autocatalytic reaction (Agrawal, 1992). A sigmoidal behavior is characterized by an initial stage where the reaction is accelerated exponentially, and a final stage where the reaction reaches its maximum at a specific value of α . It can be observed that the kinetic curves shift to the right (Figure 1a-d) as the heating rate increases. During slow heating, low molecular weight compounds formed through degradation of primary oxidation remain in solution, accelerating the degradation process. Contrary, fast heating causes the lost of such compounds through evaporation before they further react with the oil solution. The resulting net effect is that higher temperatures are needed to achieve the same oxidation degree when increasing the heating rate (Adhvaryu *et al.*, 2000; Martínez-Monteaagudo *et al.*, 2011).

The span of temperature corresponding to the oxidized fraction ($0 \leq \alpha \leq 1.0$) for MF1, MF2, MF3, and MF4 varied from 439-504, 436-522, 430-514, and 383-514 K, respectively. For all samples, the maximum heat flow temperature was below the self-ignition temperature of edible oils (~625K). Thus, the temperature span observed in Figure 1 was considered within the oxidation range. Similar oxidation temperature range has been reported for cod liver oil (Pedroza-Islas *et al.*, 2002), unsaturated fatty acids (oleic, erucic, linoleic, and linolenic acids) and saturated fatty acids (lauric, myristic, palmitic, and stearic acids) (Litwinienko *et al.*, 1999; Litwinienko 2001). The beginning of oxidation ($\alpha < 0.025$) corresponds to the start temperature of oxidation, which decreases with increasing the ratio of unsaturated to saturated fatty acids. It has been postulated that C=C serves as an active site for oxidation (Martínez-Monteaagudo *et al.*, 2011). The start temperature of oxidation for MF4 was considerable lower (~383K) compared with that of MF3 (~430K), confirming that free fatty acids oxidize at lower temperatures than fatty acids attached to a triacylglycerol backbone.

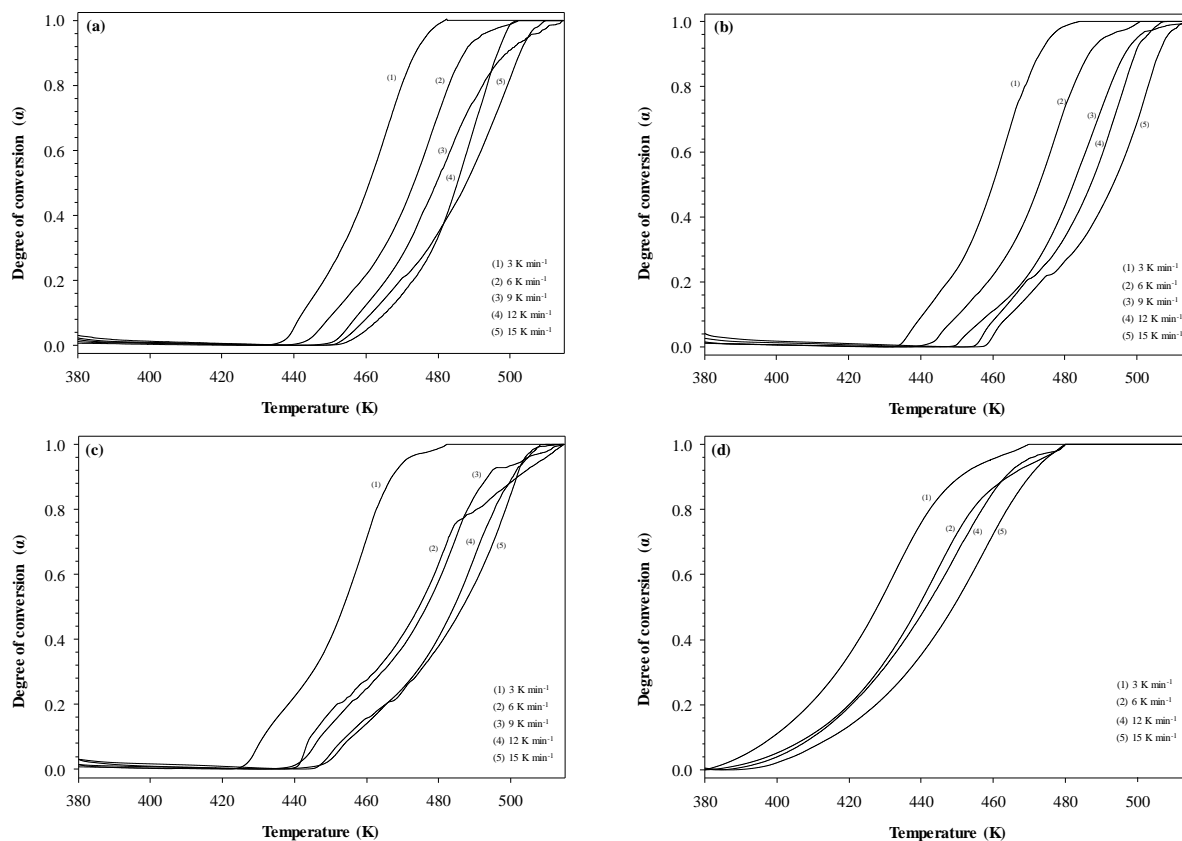


Fig. 1. Degree of conversion as a function of temperature at different heating rates for: a MF1, b MF2, c MF3, and d MF4.

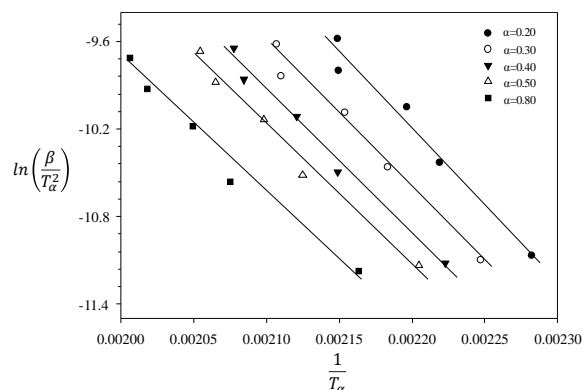


Fig. 2. Representative data showing the applicability of Kissinger-Akahira-Sunose method for the thermoxidation of milk fat.

3.2 Relationship between E_α and α

The relationship between E_α and α is evaluated through linear regression according to the KAS

method (Figure 2). Overall, the variation of E_α shows three distinctive stages (Figure 3). Stage I was observed within the range of 0.05 to 0.20 and it shows a linear decreased of E_α from 120 to 105, 105 to 96, 100 to 87, and 142 to 122 kJ mol⁻¹ for MF1, MF2, MF3, and MF4, respectively. Similarly, the thermoxidation of vinyl polymers yielded E_α values from 80-110 kJ mol⁻¹ (Vyazovkin *et al.*, 2011). In the stage II, the E_α values remained practically constant with α over 0.2 to 0.80. The stage III was considered when α varied from 0.80 to 0.95. The relationship shown in Figure 3 is used to provide an initial guess for the reaction model involved during the thermoxidation (Vyazovkin *et al.*, 2011). The decrease of E_α in the range of 0.05 to 0.20 is thought to be due to the initiation of oxidation in which numerous reactions take place including formation of radicals and primary oxidation products. The values of E_α within stage I suggest that initiations reactions are favored at high temperatures.

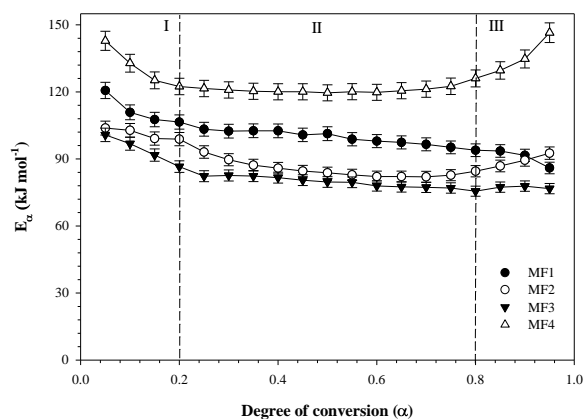


Fig. 3. Values of activation energy (E_{α}) as a function of degree of conversion (α) for the thermoxidation of milk fat containing different ratio of unsaturated to saturated fatty acids. MF1 = 0.7; MF2= 0.86; MF3= 1.36; and MF4= 1.40.

E_{α} comprised of initiations reactions that have different activation energies, the relative contributions of these reactions vary with α . The thermoxidation of milk fat within stage I ($0.05 \leq \alpha \leq 0.20$) is likely dominated by multi-step reactions. On the other hand, E_{α} was roughly constant over a large range of α (0.2 to 0.80) for all samples, indicating that the thermoxidation of milk fat is controlled by a single-step reaction, possible break down of primary oxidation products, and it can be adequately described by a single-step reaction model (Vyazovkin *et al.*, 2011). During stage II, the influence of irreversible kinetic scheme and diffusion limitations is negligible and the thermoxidation occurs via single-step irreversible scheme. Finally, the E_{α} changed with increasing α from 0.80 to 0.95. Such changes in the E_{α} might correspond when the oxidation approaching to the termination stage, where stable dimer-like products are formed (Martínez-Monteagudo *et al.*, 2011). An increasing behavior of E_{α} with respect to α have been associated with thermal decomposition of polymers such polystyrene (Shlensky *et al.*, 1988) and poly(imide siloxane) (Shi, 1990). Thermal decomposition of polymers is characterized by competition among individual molecules and intermolecular complex (Vyazovkin, 1996). Despite the three distinctive stages, the overall behavior of E_{α} toward α did not resemble the generalized behavior for multi-step reactions such competitive, consecutive, reversible, or diffusion-controlled reactions (Vyazovkin and Wight, 1997). Thus, it is reasonable to assume that the thermoxidation

of milk fat can be described by a single-step model, Figure 3. However, this interpretation must be taken cautiously because if a process is found to obey a single-step equation, one should not conclude that the process mechanism involves one single step. More likely, the oxidation mechanism involves several steps but one of them determines the overall kinetics.

3.3 Determination of reaction model

The reaction model for the thermoxidation of milk fat was determined by testing seven different models (Table 1). The fitting performance and kinetic parameters associated with the tested models are shown in Table 2-5. For all samples, the models D1, D3, and P4 failed to provide a satisfactory representation of the experimental data with R^2 values ranging from 0.101 to 0.940, depending on the heating rate. This generalization is not surprising since D1, D3, and P4 are often used for reactions controlled by diffusion (Vyazovkin, 1996). Indeed, the thermoxidation data shown in Figure 1 were generated using < 2 mg of sample in hermetic sealed pan with pinhole, a procedure that minimizes the diffusional effects (Saldaña and Martínez-Monteagudo 2013). Contrary, the first-order (F1) and the different forms of Avrami-Erofeev (A2, A3, and A4) seem to adequately represent the experimental data showing low values of RSS and high values of R^2 . The fitting performance of the different forms of Avrami-Erofeev (Table 2-5) suggested that these models are statistically appropriate to describe the thermoxidation of milk fat.

An ideal model would be one that not only having adequate statistical indexes but also providing insights into the kinetic scheme.

Table 1. Integral form of the different reaction models used in the kinetic analysis of thermoxidation of milk fat with different ratios of unsaturated to saturated fatty acids.

Reaction model	Code	$g(\alpha)$
Mampel (First-order)	F1	$1-\alpha$
One-dimensional diffusion	D1	α^2
Three-dimensional diffusion	D3	$[1-(1-\alpha)^{1/3}]^2$
Power law	P4	$\alpha^{1/4}$
Avrami-Erofeev	A2	$[-\ln(1-\alpha)]^{1/2}$
Avrami-Erofeev	A3	$[-\ln(1-\alpha)]^{1/3}$
Avrami-Erofeev	A4	$[-\ln(1-\alpha)]^{1/4}$

Table 2. Kinetic parameters and fitting performance of different reaction model for the thermoxidation of milk fat containing a ratio of unsaturated to saturated fatty acids of 0.7.

Reaction model - Mampel (F1)					
Parameter	β_3	β_6	β_9	β_{12}	β_{15}
E_a	150.6	129.3	110.84	211.52	317.18
A	2.65×10^{16}	6.51×10^{13}	4.81×10^{11}	3.11×10^{22}	1.49×10^{34}
RSS	25.43	13.24	33.11	33.67	35.81
R^2	0.979	0.993	0.983	0.976	0.973
Reaction model - One-dimensional diffusion (D1)					
Parameter	β_3	β_6	β_9	β_{12}	β_{15}
E_a	100.39	96.19	84.29	138.03	329.45
A	3.99×10^9	2.75×10^9	1.89×10^8	1.13×10^{14}	1.09×10^{34}
RSS	124.06	37.32	7.91	6.77	91.51
R^2	0.529	0.819	0.945	0.944	0.466
Reaction model - Three-dimensional diffusion (D3)					
Parameter	β_3	β_6	β_9	β_{12}	β_{15}
E_a	58.35	105.66	129.95	233.6	284.15
A	1.22×10^5	2.28×10^{10}	6.52×10^{12}	8.43×10^{23}	2.26×10^{29}
RSS	40.46	6.18	0.32	1.41	31.3
R^2	0.834	0.925	0.991	0.977	0.748
Reaction model - Power law (P4)					
Parameter	β_3	β_6	β_9	β_{12}	β_{15}
E_a	50.72	48.92	37.41	90.46	281.27
A	1.93×10^4	2.53×10^4	1.93×10^3	1.39×10^9	1.19×10^{29}
RSS	148.24	90.23	46.31	140.75	316.63
R^2	0.138	0.399	0.655	0.198	0.161
Reaction model - Avrami-Erofeev (A2)					
Parameter	β_3	β_6	β_9	β_{12}	β_{15}
E_a	178.76	124.24	110.1	208.51	318.77
A	1.90×10^{19}	9.23×10^{12}	2.01×10^{11}	7.48×10^{21}	1.11×10^{34}
RSS	3.21	3.15	8.28	8.45	8.91
R^2	0.989	0.993	0.983	0.972	0.973

Reaction model - Avrami-Erofeev (A3)					
Parameter	β_3	β_6	β_9	β_{12}	β_{15}
E_a	181.69	125.11	110.56	210.29	320.89
A	2.01×10^{19}	7.16×10^{12}	1.99×10^{11}	7.67×10^{21}	1.23×10^{34}
RSS	0.782	1.39	3.67	3.74	3.94
R^2	0.989	0.993	0.982	0.972	0.973
Reaction model - Avrami-Erofeev (A4)					
Parameter	β_3	β_6	β_9	β_{12}	β_{15}
E_a	189.69	126.56	111.74	211.38	321.63
A	2.05×10^{19}	8.22×10^{12}	1.51×10^{11}	7.51×10^{21}	1.11×10^{34}
RSS	0.78	0.79	2.07	2.1	2.21
R^2	0.989	0.993	0.983	0.973	0.975

E_a - apparent activation energy (kJ mol^{-1}); A - pre-exponential factor (K^{-1}); RSS - residual sum of squares; R^2 - coefficient of determination; $\beta_3 - 15$ - heating rate $3-15 \text{ K min}^{-1}$

Table 3. Kinetic parameters and fitting performance of different reaction model for the thermoxidation of milk fat containing a ratio of unsaturated to saturated fatty acids of 0.86.

Reaction model - Mampel (F1)					
Parameter	β_3	β_6	β_9	β_{12}	β_{15}
E_a	156.39	126.19	119.1	235.5	279.92
A	1.18×10^{17}	3.11×10^{13}	4.22×10^{12}	1.44×10^{25}	4.65×10^{29}
RSS	0.36	21.98	24.41	30.07	266.01
R^2	0.999	0.987	0.991	0.981	0.822
Reaction model - One-dimensional diffusion (D1)					
Parameter	β_3	β_6	β_9	β_{12}	β_{15}
E_a	54.4	61.48	84.33	105.39	282.9
A	4.68×10^4	5.18×10^5	1.97×10^8	4.41×10^{10}	1.51×10^{29}
RSS	55.95	21.86	10.44	7.85	17.69
R^2	0.789	0.895	0.933	0.944	0.812
Reaction model - Three-dimensional diffusion (D3)					
Parameter	β_3	β_6	β_9	β_{12}	β_{15}
E_a	56.34	102.73	133.93	198.14	286.08
A	7.47×10^4	1.14×10^{10}	2.02×10^{13}	1.91×10^{20}	1.88×10^{29}
RSS	32.85	7.64	1.16	2.89	0.17
R^2	0.859	0.944	0.974	0.964	0.993

Reaction model –Power law (P4)					
Parameter	β_3	β_6	β_9	β_{12}	β_{15}
<i>Ea</i>	15.06	18.56	22.29	43.58	233.9
<i>A</i>	1.91	9.72	37.85	1.18×10^4	1.24×10^{24}
RSS	30.26	22.2	22.85	60.36	272.01
R^2	0.805	0.845	0.837	0.477	0.182
Reaction model - Avrami-Erofeev (A2)					
Parameter	β_3	β_6	β_9	β_{12}	β_{15}
<i>Ea</i>	157.86	125.54	119.15	235.72	286.04
<i>A</i>	8.65×10^{16}	1.32×10^{13}	2.13×10^{12}	7.62×10^{24}	1.04×10^{30}
RSS	0.08	5.49	6.1	7.51	3.92
R^2	0.999	0.986	0.991	0.981	0.851
Reaction model - Avrami-Erofeev (A3)					
Parameter	β_3	β_6	β_9	β_{12}	β_{15}
<i>Ea</i>	157.69	125.74	119.14	235.46	291.13
<i>A</i>	4.13×10^{16}	9.28×10^{12}	1.42×10^{12}	4.77×10^{24}	2.42×10^{30}
RSS	0.02	2.44	2.71	3.34	1.81
R^2	0.999	0.986	0.991	0.981	0.859
Reaction model - Avrami-Erofeev (A4)					
Parameter	β_3	β_6	β_9	β_{12}	β_{15}
<i>Ea</i>	157.69	126.08	119.2	235.48	289.51
<i>A</i>	4.16×10^{16}	7.57×10^{12}	1.08×10^{12}	3.59×10^{24}	1.22×10^{30}
RSS	0.02	1.37	1.52	1.87	1.06
R^2	0.999	0.986	0.991	0.985	0.845

E_a - apparent activation energy (kJ mol⁻¹); *A* - pre-exponential factor (K⁻¹); RSS - residual sum of squares; R^2 - coefficient of determination; $\beta_3 - 15$ - heating rate 3-15 K min⁻¹

Table 4. Kinetic parameters and fitting performance of different reaction model for the thermoxidation of milk fat containing a ratio of unsaturated to saturated fatty acids of 1.36.

Reaction model - Mampel (F1)					
Parameter	β_3	β_6	β_9	β_{12}	β_{15}
<i>Ea</i>	119.12	90.62	94.16	197.35	234.24
<i>A</i>	9.27×10^{12}	2.18×10^9	8.55×10^9	1.12×10^{21}	1.03×10^{25}
RSS	20.09	49.11	30.84	83.33	113.48
R^2	0.988	0.968	0.985	0.951	0.957

Reaction model - One-dimensional diffusion (D1)

Parameter	β_3	β_6	β_9	β_{12}	β_{15}
<i>Ea</i>	34.76	64.53	67.73	91.51	235.17
<i>A</i>	352.82	9.51×10^5	3.35×10^6	1.47×10^9	1.94×10^{24}
RSS	47.99	6.28	10.37	5.65	33.53
R^2	0.823	0.955	0.983	0.959	0.736

Reaction model - Three-dimensional diffusion (D3)

Parameter	β_3	β_6	β_9	β_{12}	β_{15}
<i>Ea</i>	50.4	116.51	115.96	198.41	244.5
<i>A</i>	1.72×10^4	1.38×10^{11}	2.49×10^{11}	1.90×10^{20}	1.27×10^{25}
RSS	30.7	0.51	0.94	1.29	1.59
R^2	0.869	0.981	0.977	0.984	0.968

Reaction model - Power law (P4)

Parameter	β_3	β_6	β_9	β_{12}	β_{15}
<i>Ea</i>	13.1	16.91	19.11	16.05	183.68
<i>A</i>	1.11	6.06	16.83	8.94	9.64×10^{18}
RSS	32.49	23.54	1871	22.58	283.57
R^2	0.794	0.825	0.873	0.793	0.141

Reaction model - Avrami-Erofeev (A2)

Parameter	β_3	β_6	β_9	β_{12}	β_{15}
<i>Ea</i>	121.64	89.11	94.22	199.61	244.01
<i>A</i>	8.92×10^{12}	7.43×10^8	4.51×10^9	9.65×10^{20}	5.84×10^{25}
RSS	5.51	1.77	7.7	7.87	6.16
R^2	0.988	0.985	0.985	0.971	0.912

Reaction model - Avrami-Erofeev (A3)

Parameter	β_3	β_6	β_9	β_{12}	β_{15}
<i>Ea</i>	117.95	89.39	94.14	197.11	255.74
<i>A</i>	1.71×10^{12}	5.31×10^8	2.94×10^9	3.53×10^{20}	6.92×10^{26}
RSS	1.21	0.79	3.42	3.32	3.09
R^2	0.989	0.988	0.985	0.972	0.896

Reaction model - Avrami-Erofeev (A4)

Parameter	β_3	β_6	β_9	β_{12}	β_{15}
<i>Ea</i>	117.95	95.53	94.23	193.99	247.5
<i>A</i>	1.71×10^{12}	1.86×10^9	2.26×10^9	1.23×10^{20}	6.36×10^{25}
RSS	1.21	0.61	1.92	1.77	1.59
R^2	0.989	0.979	0.985	0.976	0.904

Ea - apparent activation energy (kJ mol^{-1}); *A* - pre-exponential factor (K^{-1}); RSS - residual sum of squares; R^2 - coefficient of determination; $\beta_3 - 15$ - heating rate $3-15 \text{ K min}^{-1}$

Table 5. Kinetic parameters and fitting performance of different reaction model for the thermoxidation of hydrolyzed milk fat containing a ratio of unsaturated to saturated fatty acids of 1.40.

Reaction model - Mampel (F1)				
Parameter	$\beta 3$	$\beta 6$	$\beta 12$	$\beta 15$
<i>Ea</i>	78.3	73.44	143.82	176.69
<i>A</i>	4.04×10^8	1.26×10^8	2.62×10^{16}	1.88×10^{20}
RSS	37.84	27.37	190.68	235.68
R^2	0.976	0.974	0.963	0.74
Reaction model - One-dimensional diffusion (D1)				
Parameter	$\beta 3$	$\beta 6$	$\beta 12$	$\beta 15$
<i>Ea</i>	19.91	26.98	42.82	180.64
<i>A</i>	7.71	96.93	1.05×10^4	1.81×10^{19}
RSS	52.81	39.26	68.16	155.3
R^2	0.783	0.841	0.729	0.209
Reaction model - Three-dimensional diffusion (D3)				
Parameter	$\beta 3$	$\beta 6$	$\beta 12$	$\beta 15$
<i>Ea</i>	33.41	70.35	147.31	189.2
<i>A</i>	248.97	5.21×10^6	1.25×10^{15}	1.42×10^{20}
RSS	39.05	45.82	150.37	59.83
R^2	0.846	0.806	0.359	0.631
Reaction model - Power law (P4)				
Parameter	$\beta 3$	$\beta 6$	$\beta 12$	$\beta 15$
<i>Ea</i>	2.37	4.19	4.98	134.57
<i>A</i>	0.01	0.09	0.26	7.94×10^{13}
RSS	15.69	15.91	19.86	561.83
R^2	0.783	0.801	0.782	0.101
Reaction model - Avrami-Erofeev (A2)				
Parameter	$\beta 3$	$\beta 6$	$\beta 12$	$\beta 15$
<i>Ea</i>	81.43	73.5	144.85	193.08
<i>A</i>	4.77×10^8	6.43×10^7	1.72×10^{16}	4.82×10^{21}
RSS	10.61	6.84	48.63	28.08
R^2	0.973	0.981	0.871	0.901

Reaction model - Avrami-Erofeev (A3)

Parameter	$\beta 3$	$\beta 6$	$\beta 12$	$\beta 15$
E_a	78.29	73.22	141.54	201.44
A	1.03×10^8	3.97×10^7	4.59×10^{15}	2.78×10^{22}
RSS	2.27	3.03	21.45	13.93
R^2	0.977	0.981	0.871	0.895

Reaction model - Avrami-Erofeev (A4)

Parameter	$\beta 3$	$\beta 6$	$\beta 12$	$\beta 15$
E_a	78.29	73.03	139.83	195.48
A	1.03×10^8	2.83×10^{17}	2.26×10^{15}	4.49×10^{21}
RSS	2.27	1.7	11.21	7.29
R^2	0.977	0.981	0.885	0.902

E_a - apparent activation energy (kJ mol^{-1}); A - pre-exponential factor (K^{-1}); RSS - residual sum of squares; R^2 - coefficient of determination; $\beta 3 - 15$ - heating rate 3-15 K min^{-1}

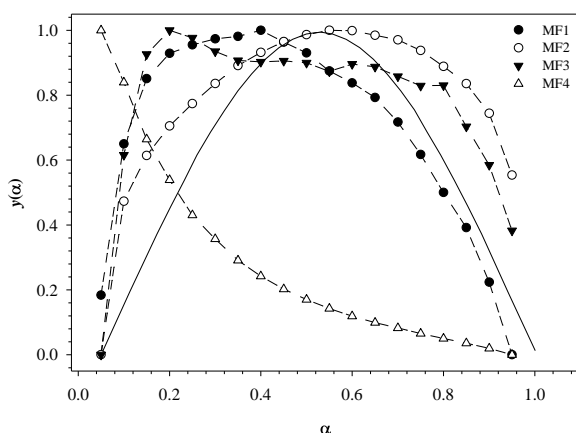


Fig. 4. $y(\alpha)$ function for the thermoxidation of milk fat. The continuous line represents the theoretical model.

A common procedure used to identify the reaction model is through $y(\alpha)$ and $z(\alpha)$ master plots, according to Equation (12) and (13).

$$y(\alpha) = \left(\frac{d\alpha}{dt} \right)_\alpha \exp\left(\frac{E_a}{RT_\alpha} \right) \quad (12)$$

$$z(\alpha) = \left(\frac{d\alpha}{dt} \right)_\alpha T_\alpha^2 \left[\frac{\pi(x)}{\beta T_\alpha} \right] \quad (13)$$

Figures 4 and 5 show the normalized $y(\alpha)$ and $z(\alpha)$ functions for all tested milk fat samples. From the shape of $y(\alpha)$ and $z(\alpha)$ functions, a suitable model is identified as the best match between experimental and theoretical functions. For MF1, MF2, and MF3, the shape of $y(\alpha)$ and $z(\alpha)$ functions resemble that of A3 model (Figure 4 and 5).

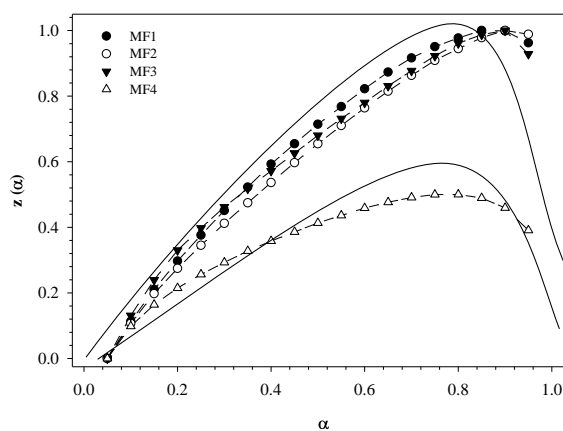


Fig. 5. $z(\alpha)$ function for the thermoxidation of milk fat. The continuous line represents the theoretical model.

In the case of MF4, $y(\alpha)$ and $z(\alpha)$ functions resemble that of a theoretical F1 model. The thermoxidation of milk fat in which the fatty acids are attached to a glycerol molecule is best described by Avrami-Erofeev model (A3). This observation contradicts the first-order assumption commonly used during the analysis of milk fat oxidation. Deviations from the first-order model are expected during the oxidation of triacylglycerol since the fatty acids are distributed throughout different triacylglycerol, resulting in different combinations of molecular weights, chain lengths, and degree of saturation (Martinez-Monteagudo and Saldana 2015).

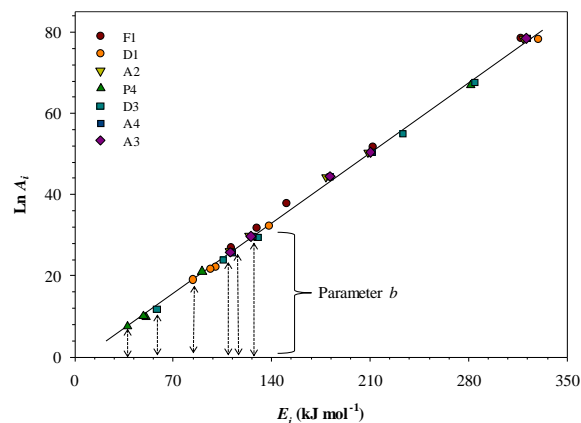


Fig. 6. Representative data showing an empirical linear relationship between pre-exponential factor and activation energy.

Interestingly, the oxidation of milk consisting of mostly free fatty acids was adequately described by a first-order model. One of the assumption of the first-order model is that the reactants are unaffected by the matrix, which is a possible scenario for free acids.

3.4 Kinetic parameters

For all samples, the A_i and E_i calculated in Tables 2-5 showed a linear relationship with the heating rate, regardless of the reaction model. Such relationship was further used explore to calculate the pre-exponential factor (A_o), according to Equation (9).

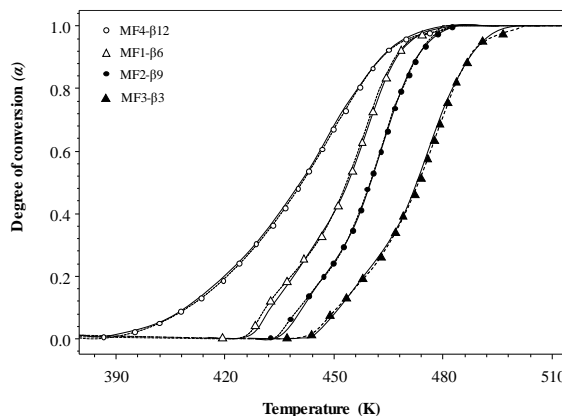


Fig. 7. Comparison of conversion as a function of temperature at different heating rates for the thermoxidation of milk fat containing different ratio of unsaturated to saturated fatty acids. The discontinue line represent the experimental data. MF1 = 0.7; MF2= 0.86; MF3= 1.36; and MF4= 1.40.

The dependence of A_i with respect to E_i for the different models was similar, and all data fell onto a master linear curve (Figure 6), where the parameters a and b were calculated through linear regression. Interestingly, the slope (parameter a) of the linear curve was the same for all models, while the parameter b which represents the intercept (arrows in Figure 6) varied depending on the model used. The pre-exponential factor was then calculated by substituting a and b into Equation (10) using the value of E_α within the α range of 0.2-0.8, where $E_\alpha \approx E_o$.

Table 6. Summary of the obtained kinetic triplets for the thermoxidation of milk fat.

Material	Kinetic triplet			
	A_o [min^{-1}]	E_o [kJ mol^{-1}]	Model code	$g(\alpha)$
MF1	1.08×10^8	100.11	A3	$[-\ln(1-\alpha)]^{1/3}$
MF2	1.17×10^7	84.53	A3	$[-\ln(1-\alpha)]^{1/3}$
MF3	1.87×10^6	80.41	A3	$[-\ln(1-\alpha)]^{1/3}$
MF4	1.60×10^{15}	121.44	F1	$(1-\alpha)$

MF1, MF2, MF3 - milk fat containing a ratio of unsaturated to saturated fatty acids of 0.7, 0.86, and 1.36, respectively; MF4 - hydrolyzed milk fat containing a ratio of unsaturated to saturated fatty acids of 1.40; A_o - pre-exponential factor; E_o - activation energy; A3 - Avrami-Erofeev model; F1 - first-order model.

Table 6 summarizes the kinetic triplet obtained for MF1, MF2, MF3, and MF4. The information presented in Table 6 was further used for modelling the thermoxidation of milk fat by substituting the kinetic triplet in Equation (6). The activation energy can be seen as the energetic barrier that fat molecules need to overcome in order to be able to react. Reactive molecules collide with a certain frequency (A), providing the energy needed to react or overcome the energetic barrier.

The frequency or pre-exponential factor (A) is also interpreted as the reaction rate when there is no energetic barrier ($E = 0$). Although milk fat is a complex system, the obtained kinetic triplet is of great relevance to describe the influence of temperature during oxidation. The resulting data (predicted) were compared with the experimental data points, Figure 7. The comparison of experimental and predicted values of the degree of conversion at increased temperature show good agreement for the respective kinetic triplet.

Conclusions

The isoconversional method was used to study the thermoxidation kinetic of milk fat containing different ratio of unsaturated to saturated fatty acids. The method revealed that the thermoxidation of milk fat was mainly controlled by single-step reaction model. The dependence of activation with respect to the degree of conversion was established. The thermoxidation of milk fat in the form of triacylglycerol was best described by the Avrami-Erofeev model, while the first-order model adequately represents the thermoxidation of free fatty acids. The obtained kinetic triplet (A_0 , E_0 , and reaction model) showed good agreement when comparing with experimental data. The outcomes of this research may enable off-line simulation and development of a databank.

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Nomenclature

CLA	conjugated linoleic acid
DSC	differential scanning calorimetry
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
KAS	Kissinger-Akahira-Sunose method
MF1	milk fat samples with a ratio of unsaturated to saturated fatty acids of 0.70
MF2	milk fat samples with a ratio of unsaturated to saturated fatty acids of 0.86
MF3	milk fat samples with a ratio of unsaturated to saturated fatty acids of 1.36
MF4	hydrolyzed milk fat sample (90% free fatty acids) with a ratio of 1.40
TVA	<i>Trans</i> -vaccenic acid
<i>Variables and constants</i>	
A	pre-exponential factor and reaction model
E_a	activation energy
E_α	activation energy at a constant degree of conversion
$f(\alpha)$	differential form of the reaction model
$g(\alpha)$	integral form of the reaction model
$k(T)$	rate constant
HF_o	initial value of the heat flow
HF_f	final heat flow
HF_T	heat flow signal at a given temperature
R	universal gas constant
R^2	coefficient of determination
R^2_{Adj}	adjusted coefficient of determination
RRS	residual analysis, and residual sum of squares
T_s	start temperature
T_p	maximum heat flow term
T_α	temperature at a constant degree of conversion
x	dimensionless activation energy
α	degree of conversion
β_i	constant heating rate

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