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ARTICLE

Kinetic Modeling of the Generation of 2- and 3-Methylbutanal in a Heated Extract of Beef Liver[§]

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Quantitative control of aroma generation during the Maillard reaction presents great scientific and industrial interest. Although there have been many studies conducted in simplified model systems, the results are difficult to apply to complex food systems, where the presence of other components can have a significant impact. In this work, an aqueous extract of defatted beef liver was chosen as a simplified food matrix for studying the kinetics of the Maillard reaction. Aliquots of the extract were heated under different time and temperature conditions and analyzed for sugars, amino acids, and methylbutanals, which are important Maillard-derived aroma compounds formed in cooked meat. Multiresponse kinetic modeling, based on a simplified mechanistic pathway, gave a good fit with the experimental data, but only when additional steps were introduced to take into account the interactions of glucose and glucose-derived intermediates with protein and other amino compounds. This emphasizes the significant role of the food matrix in controlling the Maillard reaction.

KEYWORDS: Flavor; Maillard reaction; kinetics; multiresponse kinetic modeling; 3-methylbutanal; 2-methylbutanal; beef liver

INTRODUCTION

The Maillard reaction is a complex network of parallel and consecutive reactions, initiated by the reaction between a reducing sugar and an amino compound, and is responsible for the generation of color and aroma in heated food (*1*). The aroma profile of a product influences the quality and consumer acceptance of the food; hence, control of the Maillard reaction is of particular industrial and scientific interest. Currently, it is possible to manipulate certain pathways in the Maillard reaction by altering selected precursors, but these changes are qualitative and are likely to have an impact on the wider volatile profile. Quantitative control remains a big challenge for the future, because many factors are involved, for example, reactant concentrations, time, temperature, pH, water activity, fat content, ionic strength, etc. One approach to quantitative control is the development of mathematical models, based on the reaction kinetics, which can identify the relevant rate-limiting steps and control points where the reaction can be manipulated as required (*2*).

Kinetic modeling has been used previously for studies on the Maillard reaction. Van Boekel and co-workers (*3*−*5*) have successfully modeled the Maillard reaction both in aqueous systems and in the presence of casein, and Wedzicha and co-workers have developed a three-step model that can accurately predict the formation of melanoidins in heated sugar and amino acid systems (*6*, *7*). More recently, Wedzicha proposed a kinetic model, based on the Maillard reaction, for the formation of acrylamide in low-moisture potato, wheat, and rye products (*8*) and, as a result of increased concern over the generation of this potential carcinogen in foods, other models of acrylamide formation have subsequently been published (*9*−*12*). However, none of these studies includes the formation of flavor compounds and, in general, there are only a few examples of kinetic studies containing flavor compounds (*13*).

Jousse et al. (*14*) developed a simplified kinetic model that described the formation of four classes of volatiles (pyrazines, carbonyls, pyrroles, and furans) and used existing data sets from the literature to estimate the rate constants and the activation energies for each kinetically important reaction step. They analyzed the volatiles formed from a glucose/alanine model system, heated at four different temperatures, and fitted the model to the experimental data. They stated that the proposed scheme, and the rate constants, were valid and could be used to estimate the order of magnitude of volatile generation in any Maillard system. More recently, Desclaux (*15*) used aqueous xylose/glycine and xylose/isoleucine systems to model the formation of sugar-derived reactive intermediates such as deoxyosones and dicarbonyl compounds. Van Boekel (*16*) has published a review focusing on the flavor formation during the course of the Maillard reaction. He concludes that most of the kinetics studies have been performed by using model systems of amino acids and sugars and reiterates the need for studies in real foods.

The scope of the present study was to model the aroma generation in a meat-based system, initially examining two

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important aroma compounds, 2-methylbutanal and 3-methylbutanal, which are formed during Strecker degradation, one of the many reactions that comprise the Maillard reaction. As well as being important aroma compounds, these Strecker aldehydes are also moderately reactive and can undergo condensation reactions or form heterocyclic products with important flavor characteristics in meat (*17*) and other foods (*18*).

In this study, an aqueous extract of raw defatted homogenized meat was chosen as a good base for studying the kinetics of the flavor formation during the Maillard reaction, in a system more closely resembling a real food. The use of such an extract has many advantages over the food itself: interferences from lipid and lipid interactions are removed; an aqueous system is heated more uniformly; extraction of the volatiles is simpler; yet, the system still contains many of the components present in meat at the appropriate levels. The extracts were heated under different time and temperature conditions, and the heated extracts were analyzed for sugars, amino acids, and volatile compounds. Multiresponse kinetic modeling, a well-established method for modeling complex reaction schemes, was used to generate a mathematical model of the reaction. The advantage of multiresponse modeling over single-response modeling is that all of the available experimental data are processed simultaneously to obtain good estimates for the calculated parameters and to achieve a more accurate picture for the model under study (*19*). Overall, the aim of this study was to model the generation of flavor compounds in meat-type systems using multiresponse kinetic modeling.

MATERIALS AND METHODS

Materials. Sliced ox liver was purchased from a local retailer. Four different batches of liver were used, each bought on a different date. 3-Methylbutanal (98%) was purchased from Fisher Scientific Ltd., Loughborough, U.K., and 2-methylbutanal (95%) from Alfa Aesar, Heysham, U.K. 1,2-Dichlorobenzene, L-norvaline, D-(+)-glucose, D-(−)-fructose, D-(+)-trehalose, D-(+)-maltose monohydrate, and D-(+)-mannose (all 99%) were purchased from Sigma-Aldrich Co. Ltd., Dorset, U.K.

Preparation and Heating of Meat Liver Extract. Ox liver was sliced, mixed with an equal quantity of deionized water, and homogenized for 1 min in a domestic-type blender. The slurry was then centrifuged for 20 min at 29800*g* at 4 °C and the supernatant filtered (Whatman filter no. 3) under vacuum. Aliquots of the aqueous liver extract (20 mL) were sealed in 30 mL glass ampules, immersed in an oil bath at the required temperature, and heated for different time intervals from 5 to 240 min. Batches 1, 2, and 3 were heated at 130 °C, batch 4 was heated at 120 °C, and batch 2 was split into two samples, one heated at 130 °C and the other heated at 140 °C. At 130 °C the sample took 2 min to reach 100 °C and 4 min to reach 120 °C. At least two replicates were prepared for each heating time. After heating, each ampule was immersed in a dry ice/methanol mixture at −50 °C to stop the reaction.

Analysis of Volatiles. Dynamic headspace extraction, as described previously (*20*), was used for the extraction of the volatiles. A portion of the homogenized heated extract (5 g) was mixed with HPLC grade water (10 mL) and placed in a 250 mL conical flask fitted with a Dreschel head. The flask was held in a water bath at 60 °C, and the volatiles in the headspace were swept onto Tenax absorbent using a flow of nitrogen (40 mL min^{-1}) for 1 h, followed by a purge of 100 mL min^{-1} for 10 min to remove excess solvent and moisture. GC-MS was carried out on a Perkin-Elmer Clarus 500GC-MS system (Perkin-Elmer, Beaconsfield, U.K.), equipped with an automated thermal desorber (Turbomatrix ATD), using a DB5 nonpolar column (60 m × 0.32 mm i.d., 1 μm film thickness; J&W Scientific, Agilent) under instrumental conditions described by Methven et al. (*20*). C6−C25 *n*-alkanes were analyzed under the same conditions to obtain the linear retention index (LRI) of each component. Volatile compounds were identified by comparing their mass spectra and LRIs with those of authentic compounds. Quantification of 2- and 3-methylbutanal was performed by generating calibration curves using the standard addition method and headspace collected as described above.

Sample Preparation for Free Amino Acids and Sugar Analysis. The heated liver samples were homogenized, and an aliquot (0.5 g) was mixed with 10 mL of hydrochloric acid (0.01 M) and stirred for 15 min at room temperature. The mixture was then allowed to settle for 15 min, before an aliquot of the supernatant (1.5 mL) was removed and centrifuged at 7200*g* for 30 min. It was then stored at −19 °C until further analysis.

Analysis of Free Amino Acids. The free amino acids were measured using the EZ-Faast amino acid derivatization technique (Phenomenex, Torrance, CA). A sample of the centrifuged supernatant (100 μL) was derivatized and analyzed by GC-MS on an Agilent 5975 system (Agilent, Palo Alto, CA) in electron impact mode (*21*).

Analysis of Sugars. Sugar analysis was performed using a 8220i Dionex ion chromatography system (Dionex Corp., Sunnyvale, CA) (*21*). A sample of the centrifuged supernatant (100 μL) was mixed with a solution of internal standard (900 μL of an aqueous trehalose solution (20 μg L^{-1})) and then injected onto a Carbopac PA10 column (Dionex) using an autosampler. Two different buffer solutions were prepared: A, H_2O; B, 400 mM NaOH. An isocratic program was employed for 30 min using 96% of solvent A and 4% of solvent B. For the next 10 min a solution of 40% of solvent A and 60% of solvent B was employed to wash the column. A pulsed amperometric detector was used with the following settings: 420 ms at 0.05 V, 180 ms at 0.75 V, and 420 ms at −0.15 V, and the sensitivity was set at 3K. Chromatographic analysis of peaks was performed using Chromeleon (Dionex Corp.). Standard samples of glucose, fructose, sucrose, ribose, maltose, and mannose were used for quantification.

Modeling. Multiresponse modeling was performed using the Athena Visual Studio software package (Athena Visual Software Inc., Naperville, IL).

RESULTS AND DISCUSSION

Preliminary Model. 3-Methylbutanal and 2-methylbutanal were selected for modeling because they are among the most odor-active compounds found in heated liver, and it is well-established that they are formed in the Strecker degradation of leucine and isoleucine, respectively. Although the chemistry of the Strecker degradation is well-defined, it is still complex and involves a number of intermediates of unknown concentration, some of which are very short-lived. It is, therefore, necessary to use a simplified version of the Strecker degradation pathway, using only the kinetically important steps, an approach initially proposed by Wedzicha (*22*) for an aqueous model system using glucose and glycine. In this model, the first step was a second-order reaction between glucose and glycine to form just one kinetically important intermediate, which represented a group of rate-limiting compounds. When a similar kinetic mechanism was used to build a model for the formation of 2- and 3-methylbutanal in the liver extract, the resulting model fitted the experimental data well. This model was described in earlier work (*23*) and uses the data generated from one batch of liver. However, when it was tested against the data acquired from two other batches of liver, which had different concentrations of sugars and amino acids, the model ceased to give an accurate prediction of the levels of methylbutanals formed. Therefore, the model was redesigned, and its revised form is presented in this paper.

Sugars. Glucose, mannose, and fructose were detected in all of the samples, glucose being the predominant sugar in all batches. **Figure 1a** shows the amount of glucose present as the reaction progressed. Glucose was consumed during the whole heating period, reaching levels close to zero after 240 min, indicating that it was a limiting precursor of the Maillard reaction in this meat system. It also illustrates that each batch had a different initial glucose concentration, with batch 3 having 50% more glucose than batch 1. Fructose and mannose may arise from the Lobry de Bruyn−van Ekenstein transformation (*24*), but they were present at much lower concentrations than glucose. In the raw samples, mannose was present at very low levels, whereas fructose was not

Figure 1. Changes in the levels of glucose (**a**) and amino acids (**b**–**d**) and in the formation of methylbutanals (**e**, **f**) during the course of the reaction at 130 °C for three different batches of liver extract: ∗, batch 1; □, batch 2; △ batch 3.

detected. As the reaction progressed, the concentrations of both mannose and fructose increased, reached maxima of 3.7 and 8.3 mmol kg^{-1}, respectively, and then decreased. This loss is typical of reactive intermediates; however, due to their low concentrations, they were omitted from the proposed model.

Amino Acids. Twenty free amino acids were detected in each sample. The total free amino acid content varied significantly among the three batches of liver extract (**Figure 1b**). Nevertheless, their behavior with time was similar in all three systems. During the first 20 min of heating, there was a rapid consumption of amino acids, and then their concentrations leveled off. It is likely that, although the amino acids continued to be consumed by the Strecker degradation, free amino acids were also regenerated from either the breakdown of Amadori products or proteolysis of the protein content of the meat system, resulting in no net loss or gain in the latter stages of the reaction.

Individually, leucine and isoleucine, the precursors of 3-methylbutanal and 2-methylbutanal, respectively, showed behavior similar to that of the group of total amino acids in all liver samples (**Figure 1c,d**). This implies that the ratio of leucine and isoleucine to the total amino acid content remained constant for the whole heating period. Indeed, **Figure 2** shows the linear correlation between leucine or isoleucine and the total amino acid content with R^2 values of 0.959 and 0.966, respectively. The gradients of the trend lines (0.111 and 0.034, respectively) can be used as constant values that reflect the ratio of leucine and isoleucine to the total amino acid concentration for the meat systems studied. This observation is very important for the development of the model presented in this paper.

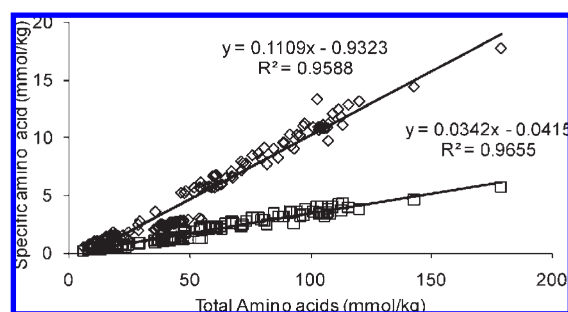

Figure 2. Relationship between leucine (◇) and isoleucine (□) concentrations and the total amino acid concentration in raw and heated liver extract.

2-Methylbutanal and 3-Methylbutanal. The results from the analysis of 3-methylbutanal and 2-methylbutanal are presented in **Figure 1e**,**f**. Their behavior is fairly typical of a reaction scheme wherein the first step is the formation of an intermediate. Formation of the product over the first 15−20 min is slow because there is no (or little) intermediate present. As the intermediate accumulates, the rate of product formation increases, and there is a period of steady growth until finally the starting material is depleted and the levels reach a maximum. Thereafter, for 3-methylbutanal, the levels started to decrease, consistent with the fact that Strecker aldehydes are not end products and they can react further to form other compounds. The same decrease was not obvious for 2-methylbutanal, and this may reflect the different reactivities of these two aldehydes.

It is also clear, from **Figure 1**, why the previous model failed when applied to all three batches of liver. The dependence on glucose is clear: the batches of liver with the highest levels of glucose produced the highest levels of Strecker aldehydes, supporting the fact that the Strecker degradation is limited by the availability of glucose. However, there was no correlation between Strecker aldehyde formation and the concentration of amino acids. Batch 2 had levels of amino acids 3−4 times higher than the other two batches, yet it did not produce significantly higher levels of Strecker aldehydes. Thus, within this range of starting materials, the rate of formation of Strecker aldehydes was independent of the amount of amino acids present. The earlier version of the model used a second-order rate equation, which appeared to describe adequately the formation of the intermediate but, with the extended data from the two further batches, the amino acid term was removed to produce a pseudo-first-order rate equation for the formation of Int1. There is also a chemical rationale for removing the amino acid term from this step. In simple aqueous model systems, in which one amino acid is used in conjunction with one reducing sugar, there is a clear dependence on the concentration of the amino acid because it is the only source of amino groups in the system. In this work, however, the reaction was carried out in a complex meat extract, which contained many other reactive amino groups such as peptides and protein-bound amino groups. Although the ratio of free amino acids to sugars was of the order of 1:1, in reality, there was an abundance of other amino groups, effectively present in excess; thus, relatively small changes in the amino acid concentration had little impact on the reaction kinetics.

Kinetic Model. After a number of possible chemical mechanisms had been considered, the reaction scheme shown in **Figure 3** was found to give the best overall fit in terms of explaining the underlying chemistry, fitting the experimental data and providing rate constants with acceptable confidence intervals. The scheme involves the formation of two kinetically important intermediates, both of which represent groups of reactive compounds. This mechanism is similar to that used previously to describe the formation of acrylamide (*8*).

Initially, glucose reacts with amino compounds to form the first group of intermediates (Int1). In this complex meat system, the amino compounds are a diverse group of compounds that include not only free amino acids and peptides with reactive α-amino groups but also proteins with reactive side chains of lysine and arginine. It can be speculated that the Int1 pool of intermediates comprises Amadori-like products and/or their breakdown products such as deoxyhexosuloses, which is supported by the studies performed by Wedzicha and co-workers (*6*, *7*). Int1 is then converted into a second kinetically important pool of intermediates (Int2) with a rate constant k_2. Int2 probably represents the

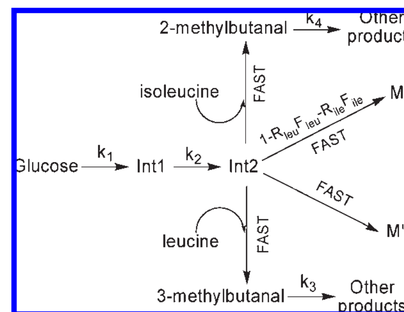

Figure 3. Proposed mechanism for the formation of 3-methylbutanal, 2-methylbutanal, and other Maillard reaction products with (M) or without (M′) the incorporation of free amino acids, respectively.

$$\frac{d[\mathrm{Glu}]}{dt} = -k_1[\mathrm{Glu}] \quad (1)$$

$$\frac{d[\mathrm{Int1}]}{dt} = k_1[\mathrm{Glu}] - k_2[\mathrm{Int1}]R_{\mathrm{leu}}F_{\mathrm{leu}} - k_2[\mathrm{Int1}]R_{\mathrm{ile}}F_{\mathrm{ile}} - k_2[\mathrm{Int1}](1 - R_{\mathrm{leu}}F_{\mathrm{leu}} - R_{\mathrm{ile}}F_{\mathrm{ile}}) - k_2[\mathrm{Int1}] \quad (2)$$

$$\frac{d[\mathrm{3MeBut}]}{dt} = k_2[\mathrm{Int1}]R_{\mathrm{leu}}F_{\mathrm{leu}} - k_3[\mathrm{3MeBut}] \quad (3)$$

$$\frac{d[\mathrm{2MeBut}]}{dt} = k_2[\mathrm{Int1}]R_{\mathrm{ile}}F_{\mathrm{ile}} - k_4[\mathrm{2MeBut}] \quad (4)$$

$$\frac{d[\mathrm{M}]}{dt} = k_2[\mathrm{Int1}](1 - R_{\mathrm{leu}}F_{\mathrm{leu}} - R_{\mathrm{ile}}F_{\mathrm{ile}}) \quad (5)$$

$$\frac{d[\mathrm{M'}]}{dt} = k_2[\mathrm{Int1}] \quad (6)$$

Figure 4. Rate equations for the reaction mechanism of **Figure 3**.

group of reactive dicarbonyl compounds that are formed in the intermediate stages of the Maillard reaction. These intermediates are very short-lived and react very quickly. At this point it is assumed that the individual rate constants for the four subsequent reactions are diffusion controlled and that the products are formed in proportion to the amount of secondary reactants present (i.e., leucine, isoleucine other amino acids, and other components, respectively). It is actually k_1 and k_2 that are rate-limiting and determine the kinetics of the formation of the final products. These rate-limiting steps are comparable to the rate-limiting steps in the model reported by Jousse et al. (*14*), who also proposed that the two intermediates were groups of sugar rearrangement products and carbonyls, respectively.

3-Methylbutanal and 2-methylbutanal are formed from the interaction of Int2 with leucine and isoleucine, respectively. Because the reaction of Int2 is very fast, it is assumed that the probability of Int2 molecules colliding and reacting with the free amino acid molecule depends on the relative concentration of the individual amino acid compared to the total amino acid concentration, and not on the absolute concentration of the amino acid. Accordingly, the amount of the reaction product is dependent on the ratio of the specific free amino acid to the total amino acid concentration. This ratio is constant, derived in **Figure 2** with values of $R_{\mathrm{leu}} = 0.111$ and $R_{\mathrm{ile}} = 0.034$ for leucine and isoleucine, respectively. This explains why there was no correlation between the amino acid content and the yield of methylbutanals in the three different batches of liver extract.

However, only a fraction of the available leucine and isoleucine was transformed into methylbutanals and, the proportion which was not was grouped with the other remaining free amino acids to form other Maillard products (M). This pathway represents all other reactions between amino acids and sugar-derived intermediates including all other Strecker aldehydes, other Maillard-derived volatiles, and melanoidins, which are colored polymers formed from sugar-containing residues and amino acids in a ratio of approximately 1:1 (*25*). This pathway accounts for a

considerable proportion of the glucose consumed in the reaction, and this was also taken into account in the earlier beef liver model (*23*). However, the loss of amino acids in all three batches is only a fraction of the loss of glucose, and about 80% of the glucose remains unaccounted for. Some of the glucose may have been converted to organic acids (*5*). This is addressed in the new model, in which an additional step has been included for the loss of glucose, without the participation of amino acids, to form a group of products (M′). In chemical terms, it is suggested that glucose, and any of the compounds in either Int1 or Int2, can react with protein. Although this reaction can happen at all three points in the proposed reaction scheme, it has been assumed that Int2, which contains very reactive dicarbonyl species, is more reactive than Int1 or glucose, and just the one additional reaction step has been incorporated into the new model. Inclusion of this step acknowledges the fact that much of the glucose is bound to the soluble protein. This reaction is analogous to the reaction attracting much attention in the medical world, where it has been shown that intermediates of the Maillard reaction, particularly the dicarbonyl compound methylglyoxal, react with proteins in vivo (*1*).

This revised reaction pathway was used to generate the rate equations in **Figure 4**. Glucose reacts with amino compounds at a rate governed by k_1. By assuming that the concentration of the amino compounds remains virtually constant during the heating period, due to the high protein content in our system and the regeneration of the free amino acids, the whole reaction can be expressed by a pseudo-first-order rate equation. Thus, differential eq 1 is the mathematical expression that describes the behavior of glucose in our system. Int1 is converted to Int2 through a reaction with rate constant k_2, and the corresponding mathematical equation is eq 2. The formation of the final products is dependent on the rate constant k_2 because Int2 reacts very quickly. This is reflected in the rate equations that describe the formation of the various products. The mathematical expressions for the formation of 3-methylbutanal and 2-methylbutanal are eqs 3 and 4, respectively. For 3-methylbutanal, k_2 is multiplied by the concentration of Int1, by the ratio R_{leu} (the ratio of the specific free amino acid to the total amino acid concentration), and by the fit constant F_{leu}, which expresses the proportion of the reacted leucine that is converted to 3-methylbutanal. The same applies for 2-methylbutanal with R_{ile} and F_{ile}, the ratio to the total amino acids and the fit constant for isoleucine, respectively. The formation of the other Maillard products (M) is expressed by eq 5. The factor $(1 - R_{leu}F_{leu} - R_{ile}F_{ile})$ expresses the probability that all of the amino acids, except the fraction of leucine and isoleucine that has been converted to 3- and 2-methylbutanal, react with Int2 to form other compounds (M). The formation of products in reactions in which the free amino acids do not participate is expressed by eq 6.

To calculate the activation energies of the reactions, a portion of liver extract from batch 2 was heated at 140 °C. Furthermore, extract from a fourth batch of liver was prepared and heated for different time intervals at 120 °C. The Arrhenius equation was reparametrized as described by Brands and van Boekel (*3*) and was employed in conjunction with the equations in **Figure 4**. The revised mathematical model contained 10 parameters (k_1, k_2, k_3, k_4, E_{a1}, E_{a2}, E_{a3}, E_{a4}, F_{leu}, F_{ile}), which were estimated using the five sets of data that were described earlier, using the Athena Visual Studio software. The results of the parameter estimation are illustrated in **Table 1**. The quality of the fit of this revised model with the experimental data is shown in **Figure 5**.

The estimates for k_1 and its corresponding E_{a1}, as well as for the fit constants F_{leu} and F_{ile}, are all good with percentage confidence intervals of ≤11%. The values of the fit factors F_{leu} and F_{ile} suggest that <5% of the reacted leucine and isoleucine was converted into methylbutanals and the remainder was converted into melanoidins and other products. Although the confidence interval for E_{a2} was very high, the value (48.7 kJ mol^{-1}) was comparable to that found by Jousse et al. (*14*) (66.5 kJ mol^{-1}) in a similar reaction scheme, but a different model system. By comparison, Cremer and Eichner (*26*) applied zero-order kinetics and calculated the activation energies of the formation of 3-methylbutanal and 2-methylbutanal to be rather higher (120 and 124 kJ mol^{-1}, respectively) in low-moisture model systems. Chan and Reineccius (*27*) performed studies in simple aqueous glucose/amino acid model systems. They applied pseudo-zero-order kinetics and calculated the activation energy of 3-methylbutanal formation to be 80.4 kJ mol^{-1}.

Table 1. Optimal Estimates for the Parameters in the Revised Model (**Figure 3**)

parameter	optimal estimates[a]
k_1[b] (min^{-1})	$1.36 \times 10^{-2} \pm 9.22 \times 10^{-4}$ (7%)
k_2 (min^{-1})	$5.77 \times 10^{-2} \pm 2.88 \times 10^{-2}$ (50%)
k_3 (min^{-1})	$2.72 \times 10^{-3} \pm 1.01 \times 10^{-3}$ (37%)
k_4 (min^{-1})	$2.56 \times 10^{-4} \pm 8.75 \times 10^{-4}$ (342%)
F_{leu}	$2.33 \times 10^{-2} \pm 2.45 \times 10^{-3}$ (11%)
F_{ile}	$3.83 \times 10^{-2} \pm 4.28 \times 10^{-3}$ (11%)
E_{a1} (kJ mol^{-1})	$1.37 \times 10^{2} \pm 1.52 \times 10^{1}$ (11%)
E_{a2} (kJ mol^{-1})	$4.87 \times 10^{1} \pm 8.40 \times 10^{1}$ (173%)
E_{a3} (kJ mol^{-1})	$7.82 \times 10^{1} \pm 3.90 \times 10^{1}$ (50%)
E_{a4} (kJ mol^{-1})	not determined

[a] Optimal values of the parameters and their 95% higher posterior density intervals. Values in parentheses are the percentage confidence intervals. [b] Rate constants are at 130 °C.

The value of k_3 confirms that there is indeed some degradation of 3-methylbutanal and that during prolonged heating, the levels will decrease. The worst estimate was k_4 (for which the confidence interval was much bigger than the value) and its corresponding E_{a4}, for which the software was unable to estimate a value. This is not surprising because it is not clear, from the experimental data, whether there was indeed any subsequent degradation of 2-methylbutanal (**Figure 1f**).

The model was based on kinetic models already reported for aqueous model systems with two key modifications. The first was removal of the amino acid concentration from the first step of the Maillard reaction. It is suggested that in a complex meat system, there are many other sources of amino groups that can participate in the reaction, such that small changes in free amino acid concentration have a minimal effect on the overall rate of the early stages. The second modification was inclusion of a step that represents the irreversible reaction between glucose and protein or other amino compounds. This was included because up to 80% of the initial glucose remained unaccounted for using the original model and has been previously described by van Boekel and collaborators (*3*, *5*). These two modifications are indicative of the problems that arise when the Maillard reaction is modeled in a real food system and reinforce the fact that, although very useful information can be gained from studying aqueous model systems, the complexity of real food systems has a major influence on the reaction kinetics.

The model presented in this paper is system dependent and has not yet been challenged by using a more diverse range of starting conditions. Furthermore, leucine and isoleucine are incorporated in the model indirectly, by using their ratio to the total amino acid amount, and the validity of the model has not been tested using meat systems with different leucine and isoleucine ratios. Finally, the model contains five "black boxes". They are the unidentified and nonquantified products of the reaction under study, which

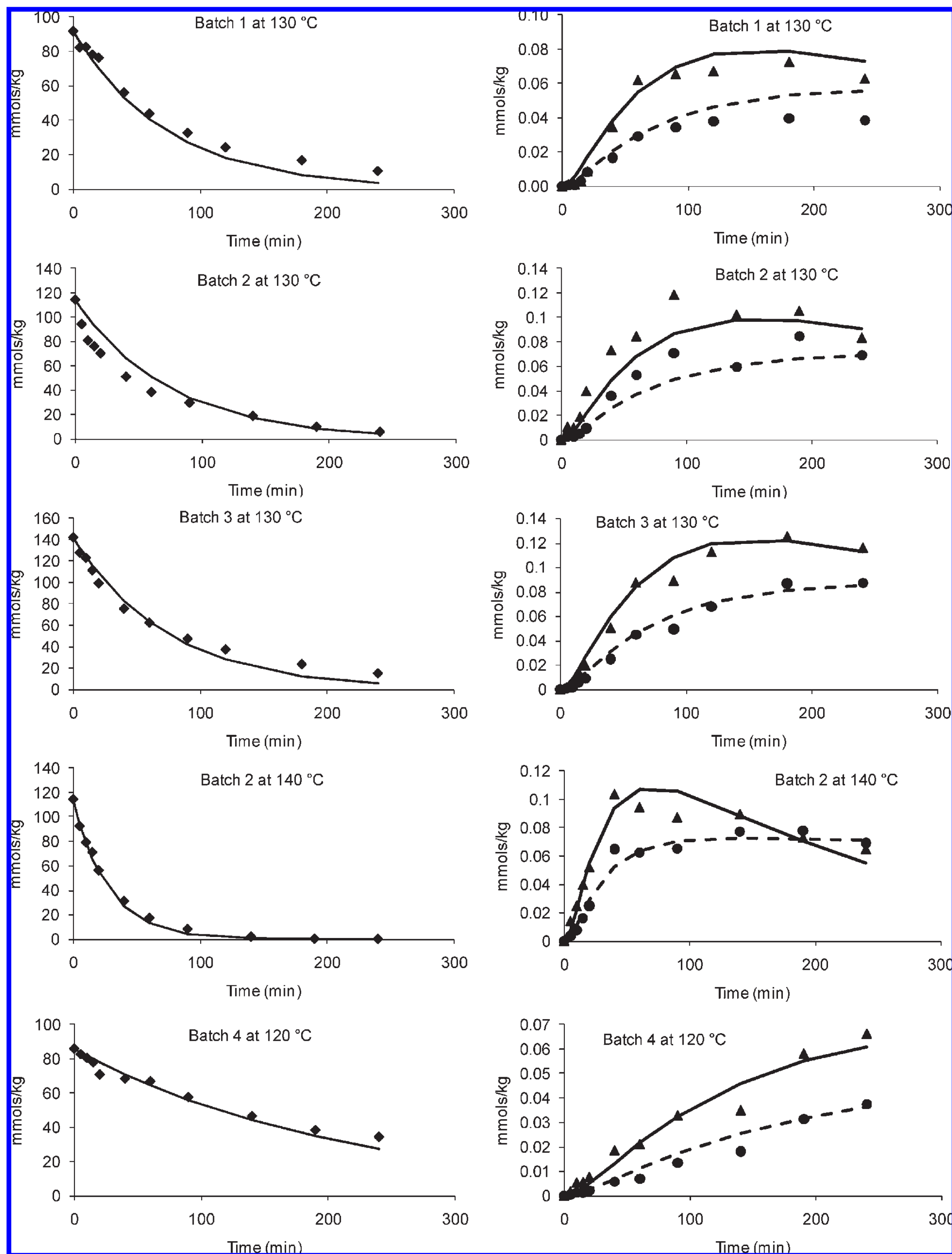

Figure 5. Overlay of the experimental data (points) and the predicted values from the revised model (lines) for glucose (◆ and —), 3-methylbutanal (▲ and —), and 2-methylbutanal (● and - - -).

give the model more flexibility to fit the experimental data. These products should be characterized and quantified to obtain more confidence in the proposed model.

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