3

Kinetic modelling

**M. A. J. S. van Boekel, Wageningen University, and
L. M. M. Tijskens, ATO-BV, Wageningen**

3.1   Introduction

Kinetic modelling is a technique that is very useful in relation to food processing and food quality. The reason is twofold. First, changes in foods as a result of processing and storage lead to a change in quality (usually a quality loss). The processes involved are mainly (bio)chemical and physical reactions. Such changes proceed at a certain rate and with certain kinetics. Kinetic modelling enables us to describe these changes and their rates quantitatively. Second, with kinetic modelling we have a powerful tool that can help to unravel basic reaction mechanisms. The understanding of the basic mechanisms is vital for quality modelling and quality control.

To understand the progress of reactions, knowledge of thermodynamics and kinetics is required. Thermodynamics is helpful in describing and understanding in which direction a reaction will proceed and the energy and entropy changes that are involved. Thermodynamics thus explains the driving force for a reaction. However, thermodynamics cannot tell anything about the speed at which a reaction proceeds. This is the domain of kinetics. The rate with which a reaction proceeds is the resultant of the driving force and the resistance against change. There is thus an intimate link between thermodynamics and kinetics.

The field of chemical kinetics originated in the second half of the nineteenth century, for instance by scientists such as Arrhenius and van 't Hoff. In the early twentieth century Ball,[1] Stumbo[2] and Bigelow pioneered kinetic principles in food processing (mainly the canning industry). They introduced parameters such as the D-value (decimal reduction time, a measure for reaction rates) and the z-value (a parameter characterizing temperature sensitivity). These D- and z-value parameters are still used by industry and legislation, despite the known deviance

from reality. The use of kinetics is not limited to (bio)chemical reactions; it is also applicable to microbiological as well as physical changes such as crystallization, aggregation and coalescence.[3]

Nowadays numerical procedures are so reliable and computers so fast that it has also become possible to model very complicated reactions in foods. A basic rule in modelling should, however, not be forgotten, namely that a model should be as simple as possible ('Ockham's Razor'),[4] but nevertheless comply with the occurring processes. So, even in the era of computer-aided modelling, models should not be overparameterized. Kinetics has developed into a powerful tool for modelling of quality attributes in foods. Nevertheless, (bio)chemical and physical insight is a prerequisite for correct application of food quality modelling and kinetic parameters have to be extracted from experiments to calibrate the models. It is thus of utmost importance to establish a fundamental reaction mechanism and to derive kinetic parameters in the most accurate way. Correct application of kinetic principles is essential. In this chapter we will go deeper into these principles in relation to food and food processing.

3.2   Key principles and methods

3.2.1   Simple kinetics

Chemical reactions are basically monomolecular or bimolecular, very rarely termolecular. A monomolecular reaction results from an internal change in a molecule, and a bimolecular reaction is the resultant from two interacting molecules. The fundamental principles of kinetic modelling are based on the conversion of chemical reaction mechanisms into the constituting differential equations, applying the law of mass action. The rules of this conversion can be found in any good textbook on chemical or enzyme kinetics.[5–8]

The most simple example is an irreversible first order (monomolecular) decay or conversion reaction:

$$A \xrightarrow{k} B \tag{3.1}$$

This mechanism results in the following set of differential equations.

$$\frac{d[A]}{dt} = -k[A]$$

$$\frac{d[B]}{dt} = k[A] \tag{3.2}$$

Zero-order formation, where a product B is formed out of reactant A present in excess, can be represented by the same reaction mechanism:

$$A \xrightarrow{k} B \tag{3.3}$$

The constituting differential equation, assuming an excess and therefore constant concentration of component A, is:

$$\frac{d[B]}{dt} = k[A] = k' \tag{3.4}$$

with k' the (pseudo) zero order rate constant; the rate is seen effectively to be independent on concentration of B. The rate will, however, depend on the constant concentration of A. In this model simplification, already a steady-state approximation is applied to the concentration of component A.

For an irreversible second order (bimolecular) reaction the following representation applies:

$$2\,A \xrightarrow{k} B \tag{3.5}$$

with the constituting differential equations:

$$\frac{d[A]}{dt} = -2k[A]^2$$

$$\frac{d[B]}{dt} = k[A]^2 \tag{3.6}$$

All these sets of differential equations can be solved easily at constant external conditions (mainly temperature and pH).

3.2.2 Complex kinetics

Reversible reactions are already more complex. Suppose we have the following reversible reaction (not yet at equilibrium)

$$A \underset{k_2}{\overset{k_1}{\rightleftarrows}} B \tag{3.7}$$

The differential rate equations are:

$$\frac{d[A]}{dt} = -k_1[A] + k_2[B]$$

$$\frac{d[B]}{dt} = k_1[A] - k_2[B] \tag{3.8}$$

The integrated rate equations, assuming an initial concentration of $A = A_0$ and of $B = 0$, are:

$$[A] = \frac{[A]_0}{k_1 + k_2}\left(k_2 + k_1 e^{-(k_1+k_2)t}\right)$$

$$[B] = \frac{[A]_0 k_1}{k_1 + k_2}\left(1 - e^{-(k_1+k_2)t}\right) \tag{3.9}$$

At a certain stage, the rates for the forward and the reverse reaction become equal, equilibrium is reached and the equilibrium constant K_{eq} is given by equation (3.10):

$$K_{eq} = \frac{k_1}{k_2} = \frac{[B]}{[A]} \tag{3.10}$$

An example of a reversible reaction relevant for foods is the mutarotation of reducing sugars.

Even more complex reactions are consecutive and parallel reactions. Consecutive reactions are reactions in which products are formed as intermediates, which then react further. The simplest example is:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \tag{3.11}$$

The differential rate equations for this case are:

$$\frac{d[A]}{dt} = -k_1[A]$$

$$\frac{d[B]}{dt} = k_1[A] - k_2[B]$$

$$\frac{d[C]}{dt} = k_2[B] \tag{3.12}$$

The analytical solution at constant external conditions or integrated rate equations are:

$$[A] = [A]_0 e^{-k_1 t}$$

$$[B] = [B]_0 e^{-k_2 t} + k_1 [A]_0 \frac{e^{-k_1 t} - e^{-k_2 t}}{k_2 - k_1}$$

$$[C] = [C]_0 + [B]_0 (1 - e^{-k_2 t}) + [A]_0 \left(1 + \frac{k_1 e^{-k_2 t} - k_2 e^{-k_1 t}}{k_2 - k_1} \right) \tag{3.13}$$

Figure 3.1 gives an example of a (hypothetical) consecutive reaction $A \rightarrow B \rightarrow C$ at various temperatures, assuming an activation energy of 5000 for the step $A \rightarrow B$ and 15000 for $B \rightarrow C$ (see section 3.2.5 for a further discussion on activation energy).

Parallel reactions imply that a reactant is subject to two or more different elementary reactions at the same time:

$$A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} P \tag{3.14}$$

$$A \longrightarrow Q$$

The differential equation for component A is

$$-\frac{d[A]}{dt} = k_1[A][B] + k_2[A] \tag{3.15}$$

but an analytical solution for the integrated rate equation is not found easily; one has to resort to a numerical solution in this case. An example of such a parallel
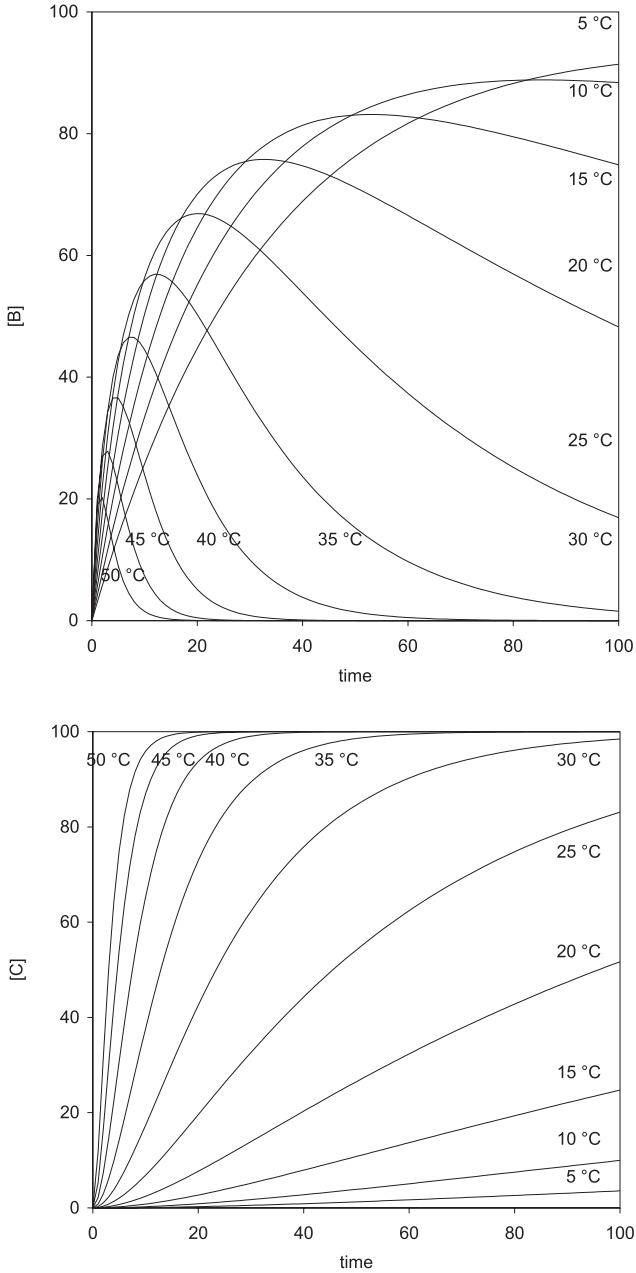
Fig. 3.1   Time course of a hypothetical consecutive reaction $A \to B \to C$ for components B and C as a function of temperature with $E_a = 5000$ for the first reaction and $E_a = 15000$ for the second reaction (arbitrary units)

reaction in foods is the simultaneous isomerization of glucose and its participation in the Maillard reaction.[9] Maillard reactions may occur during sterilization of foods and result in colour and flavour changes.

There are many examples of parallel and consecutive reactions in foods, for instance, again, the Maillard reaction,[9] or degradation of chlorophyll during heating.[10] Another practical example is the change in activity of the enzyme polygalacturonase (PG) during storage.[11] Parallel and consecutive reactions are particularly amenable to multiresponse analysis, which has distinct advantages, as discussed in section 3.2.7.

Even for relatively simple cases, the analytical solutions of the constituting differential equations at constant conditions can be quite complicated. Many more possibilities of complex reactions exist. However, for more complex reactions than the ones given, it will be very tedious, if not impossible (for instance in cases such as equation (3.15), to derive analytical solutions. The only option that is left is numerical integration of the differential equations. Fortunately, this is no problem any more with modern computers and software.[12–16]

Bimolecular reactions require that molecules will have to come together before they can interact. Encounters may occur due to temperature-induced movement, diffusion and flow. If particles would react immediately upon an encounter, the rate of reaction is controlled by diffusion; such reactions are called diffusion controlled. On theoretical considerations, one can deduce that for the fastest bimolecular reaction rate possible in water at 20ºC the reaction rate constant is $6.6 \times 10^9 \, dm^3 \, mol^{-1} \, s^{-1}$ and at 100ºC $3 \times 10^{10} \, dm^3 \, mol^{-1} \, s^{-1}$. These should be roughly the upper limits for bimolecular reaction rates at the temperature indicated.[3] For monomolecular dissociation in solution, the rate is determined by the rate at which the products can diffuse away. The upper limit for monomolecular reaction rate constants (uncharged reactions)[3] would then be roughly $10^{12} \, s^{-1}$.

3.2.3   Steady-state approach

In the literature, one frequently approximates kinetic equations for consecutive reactions by assuming the so-called steady-state, or quasi-steady-state approximation (QSSA). In the above example of the consecutive reaction mechanism (equation (3.13)), intermediate B could be very reactive and have a fast turn-over rate. This effectively comes down to the situation that after some initial induction period, $d[B]/dt \approx 0$. Such an assumption greatly simplifies the resulting rate equations, and that is the very reason for introducing steady-state assumptions. Another advantage of a steady-state approximation is that it gives a 'feel' for the most important steps, and probably makes it more comprehensible. QSSA could also be helpful in 'mechanism reduction', i.e. reduction of the number of species and therefore reduction in the number of differential equations. To achieve this, it is necessary to identify reactants and products as important (those for which accurate calculation of concentrations is the aim), as necessary (those which are necessary to calculate the concentrations of the

important ones), or as redundant (those that can be omitted without appreciable effect on the reaction network). However, a steady-state approach should not really be necessary any more, because, as mentioned above, differential equations can be solved easily by numerical integration, should analytical integration appear to be impossible. Steady-state assumptions need not be made any more in this computer age, at least not from the standpoint that rate equations can otherwise not be derived any more. Some examples of software packages that work with differential rate equations rather than integrated rate equations are given in Stewart et al.[15] and Kuzmic.[16]

3.2.4   Enzyme kinetics

Enzyme kinetics are an example where the steady-state approach is used, namely in the famous Michaelis-Menten equation. Michaelis-Menten kinetics accounts for the kinetic properties of many enzymes but certainly not all. It provides, however, more a line of reasoning to deduce possible mechanisms and to develop useful models than a prescription of the mechanism. It is the most simple approach to enzyme kinetics. A relation is sought between the rate of product formation (rate of catalysis) and the concentration of enzyme and substrate. The development of Michaelis-Menten kinetics as it is used today was actually due to more researchers than Michaelis and Menten.[8] The first proposal came from Henry, later refined by Michaelis and Menten who assumed the establishment of a rapid equilibrium between enzyme E and substrate S leading to the formation of an enzyme-substrate complex ES. The active complex ES is then converted into product P liberating the enzyme E again:

$$\text{E} + \text{S} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{ES} \xrightarrow{k_3} \text{E} + \text{P} \tag{3.16}$$

(Equation (3.16) describes, in fact, a consecutive reaction, discussed above.) There is also the Van Slyke equation, which differs from equation (3.16) in that the formation of the ES complex is assumed to be irreversible ($k_2 = 0$). It leads eventually to the same equation but with a slightly different meaning of the constants involved. Another assumption was that products do not revert to substrate, so the reaction E+P does not result in ES. That means that the rate of product formation v is:

$$v = k_3[\text{ES}] \tag{3.17}$$

which is a 'normal' first-order rate process. Later, Briggs and Haldane[8] introduced the assumption that the rate of formation of the ES complex equals that of its breakdown (steady-state assumption):

$$k_1[\text{E}][\text{S}] = (k_2 + k_3)[\text{ES}] \tag{3.18}$$

and hence an expression for [ES] is:

$$[ES] = \frac{[E][S]}{(k_2 + k_3)/k_1} \tag{3.19}$$

The Michaelis constant K_M is now introduced:

$$K_M = \frac{k_2 + k_3}{k_1} \tag{3.20}$$

and equation (3.19) can be written as:

$$[ES] = \frac{[E][S]}{K_M} \tag{3.21}$$

Meanwhile, the total concentration of the enzyme ($[E_T]$) can be written as:

$$[E_T] = [E] + [ES] \tag{3.22}$$

Substituting for [E] in equation (3.21) and solving for [ES] gives:

$$[ES] = [E_T]\frac{[S]/K_M}{1 + [S]/K_M} = [E_T]\frac{[S]}{[S] + K_M} \tag{3.23}$$

Combining equation (3.23) with equation (3.17) gives:

$$v = k_3[E_T]\frac{[S]}{[S] + K_M} \tag{3.24}$$

The expression $k_3[E_T]$ represents the maximal rate v_{max}, namely when [S] is much greater than K_M and consequently $[S]/([S]+K_M)$ in equation (3.23) becomes unity so that:

$$v_{max} = k_3[E_T] \tag{3.25}$$

Hence, equation (3.25) can be written as:

$$v = v_{max}\frac{[S]}{[S] + K_M} \tag{3.26}$$

and this is the famous Michaelis-Menten equation. Equation (3.26) describes the hyperbolic curve for the relation between rate v and [S] that is found with many (but certainly not all) enzymes (Fig. 3.2). The physical significance of K_M is that it represents the substrate concentration at which $v = 0.5v_{max}$.

Some interesting features follow from equation (3.26). When $[S] >> K_M$:

$$v = v_{max} \tag{3.27}$$

and the result is a zero-order reaction for this condition. On the other hand, when $[S] << K_M$:

$$v = [S]\frac{v_{max}}{K_M} \tag{3.28}$$

Since v_{max} and K_M are constants, the rate is directly proportional to [S], i.e. a first-order reaction appears under this condition.
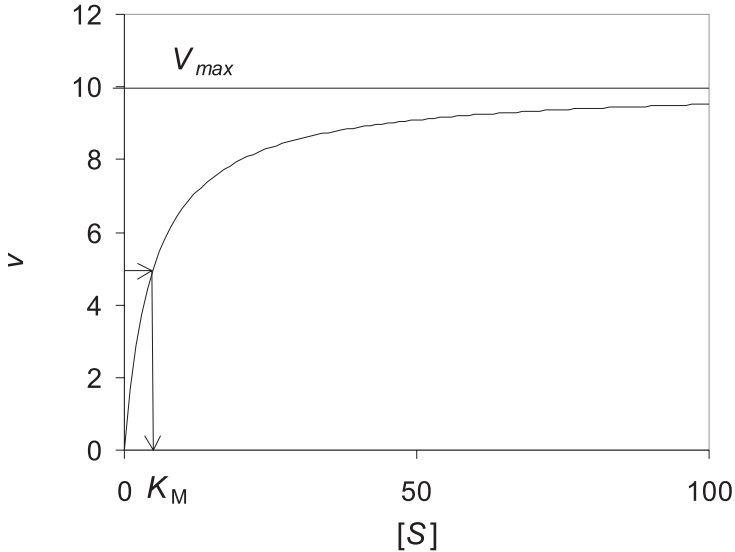
Fig. 3.2   Graphical depiction of Michaelis-Menten kinetics (arbitrary units).

3.2.5   Effects of temperature

Many foods are, for various reasons, heat treated or stored in chilled rooms and the effect of heat treatment on food quality is a very important issue. Temperature is also of importance with regard to keeping quality[23] or shelf-life. In general, temperature is the most important of all external factors. Therefore, knowledge of how the kinetics of reactions are affected by temperature is essential. Most food scientists would tend to use Arrhenius' law and derive activation energy from it. Arrhenius derived his equation empirically. Later on it was put into a theoretical perspective, especially for gas reactions, based on the collision theory, which incorporates time via molecular velocities and the number of favourably-oriented high-energy collisions (kinetic theory of gases). Arrhenius' equation appears to fit many reactions and is therefore used frequently. Although it may be a perfectly good choice in many cases (but not all), it seems appropriate to start at a somewhat more fundamental level by explaining relevant aspects of transition state theory, also referred to as the activated complex theory or absolute rate theory. This theory bridges the gap between thermodynamics and kinetics by postulating an equivalence between energy E and frequency of atomic motions ν, making it possible to deduce rate data from energy data (using the Planck expression $E = h\nu$, with h Planck's constant).

The transition state theory forms a theoretical basis to deduce more practical equations (such as Arrhenius' law). The theory is well suited for reactions in solutions and is not concerned with rates of encounters (like in gas reactions) but considers thermodynamic and statistical mechanics principles to predict how

many combinations of reactants will be present in the so-called transition state. This is a type of high-energy state in which molecules can be present in an unstable but activated condition; they will either turn back into reactants or undergo some molecular change to return as products. Consider the reactants A, B that are transformed into products P, Q via a transition state $AB^{\ddagger}$, as follows,

$$A + B \underset{\longleftarrow}{\longrightarrow} AB^{\ddagger} \rightarrow P + Q \tag{3.29}$$

Figure 3.3 shows schematically how the potential energy of the system changes with the reaction coordinate, i.e., the path along the potential energy curve. The reaction coordinate indicates the state of the molecules in the transition from reactants to products. When they start to interact the potential energy increases, and a maximum is reached in the activated, or transition state. It decreases again when products are formed.

The first step is considered to be a (quasi) equilibrium between the transition state and the reactant molecules, characterized by a practical equilibrium constant $K^{\ddagger}$ with dimension concentration^{-1} (hence not a thermodynamic one)

$$K^{\ddagger} = \frac{[AB^{\ddagger}]}{[A][B]} \tag{3.30}$$

The rate at which this equilibrium is established is fast compared to the rate of conversion of $AB^{\ddagger}$ to P, Q, so the position of the equilibrium is not perturbed
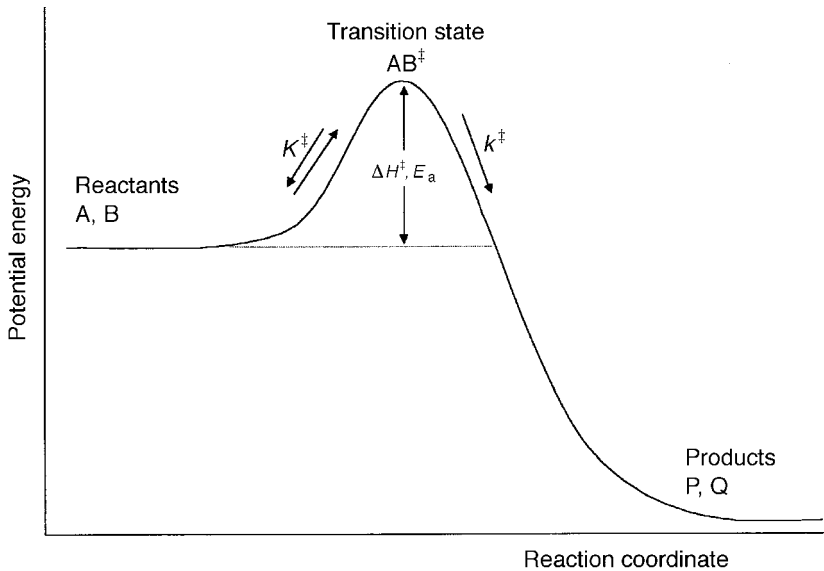

Fig. 3.3   Schematic presentation of the potential energy of reactants A, B, transition state $AB^{\ddagger}$ and products P, Q along the reaction coordinate. The energy barrier is the activation enthalpy $\Delta H^{\ddagger}$ (in the case of transition state theory) or the activation energy E_a (in the case of Arrhenius' equation).

significantly. To be explicit: $[AB^{\ddagger}]$ is not an intermediate that can be isolated, because of its instability. The second step, the formation of products, is considered to be unimolecular with a rate constant $k^{\ddagger}$ without any barrier, and considerations based on statistical mechanics result in

$$k^{\ddagger} = \frac{k_B T}{h} \tag{3.31}$$

in which k_B is Boltzmann's constant ($1.3807 \times 10^{-23}\,\mathrm{J\,K^{-1}}$), h is Planck's constant ($6.626 \times 10^{-34}\,\mathrm{J\,s}$) and T the absolute temperature (K). $k^{\ddagger}$ has dimension of frequency ($\mathrm{s^{-1}}$). The rate of formation of products is thus

$$\frac{\mathrm{d}[P]}{\mathrm{d}t} = k^{\ddagger}[AB^{\ddagger}] = \frac{k_B T}{h}[AB^{\ddagger}] = \frac{k_B T}{h} K^{\ddagger}[A][B] \tag{3.32}$$

Comparing equation (3.32) with a 'normal' rate equation for a bimolecular one shows that the rate constant k can be expressed as

$$k = \frac{k_B T}{h} K^{\ddagger} \tag{3.33}$$

The equilibrium constant relates to the Gibbs energy, and consequently the enthalpy and entropy, of activation, as follows,

$$\frac{K^{\ddagger}}{(c^{\theta})^{1-m}} = e^{\frac{\Delta G^{\ddagger}}{RT}} = e^{\frac{\Delta S^{\ddagger}}{R}} e^{\frac{-\Delta H^{\ddagger}}{RT}} \tag{3.34}$$

The factor $(c^{\theta})^{1-m}$ is necessary to turn the practical equilibrium constant $K^{\ddagger}$ into a thermodynamic one, i.e., dimensionless. c^{θ} is the concentration in the standard state, usually chosen as mol dm^{-3}, and m is the molecularity (in this case 2, see equation (3.29)). Combination of equations (3.33) and (3.34) then gives

$$k = \frac{k_B T}{h} e^{\frac{-\Delta S^{\ddagger}}{R}} e^{\frac{\Delta H^{\ddagger}}{RT}} (c^{\theta})^{1-m} \tag{3.35}$$

Equation (3.35) has the correct units for a rate constant of any order because of the factor $(c^{\theta})^{1-m}$, the concentration in the standard state to which the thermodynamic parameters are referred. This equation is referred to as the Eyring equation, after one of the developers of the transition state theory. The importance of this equation is that it relates the effect of temperature on the reaction rate constant to fundamental terms of enthalpy and entropy changes. If, for instance, a high enthalpy of activation exists, this would make the reaction quite slow at moderate temperatures, but this may be compensated by an increase in activation entropy such that the reaction can still proceed at a measurable rate. A striking example of such a phenomenon is the unfolding of proteins. This indeed requires a high activation enthalpy because of the high number of bonds being broken simultaneously upon unfolding but, at the same time, the entropy of the unfolded chain increases enormously. In other words, high activation

enthalpies *and* entropies are characteristic for protein unfolding. On the other hand, bimolecular reactions usually have a negative activation entropy (because of bond rearrangements and bond formation, entropy of the two reactants is lost), and a moderate activation enthalpy (breaking old bonds will release energy, but forming new ones will cost energy). Unimolecular reactions are usually characterized by a moderate activation entropy (either slightly negative or positive, depending on intramolecular changes, the exception being protein unfolding) and an activation enthalpy depending on the type of mechanism.

The activation enthalpy and entropy are usually assumed to be independent of temperature, which in general is probably not true, but for the heat treatment of foods the temperature range is mostly not so large, so the approximation may hold. A notable exception is, again, protein unfolding in an aqueous environment, because interaction with water comes into play. Upon unfolding, hydrophobic groups are exposed and cause increased structuring of water. There is thus also a contribution of enthalpy and entropy changes of the solvent water which may oppose the positive enthalpy and entropy for protein unfolding. The ordered solvent structure around hydrophobic groups is broken down as temperature increases. Hence the difference in heat capacity between unfolded and folded (native) proteins is quite large, resulting in temperature dependency of (activation) enthalpy and entropy.

Arrhenius' law was derived empirically, but it found its roots in the kinetic theory of gases. It has proven to be very worthwhile in chemical kinetics. Arrhenius' law states that

$$k = A e^{-\frac{E_a}{RT}} \tag{3.36}$$

in which A is the so-called pre-exponential factor (sometimes also called the frequency factor), and E_a the activation energy. It is very instructive to compare Arrhenius' law equation (3.36), with the expression derived from transition state theory equation (3.35). The dimension of A should be the same as that of the rate constant k; it therefore does have units of frequency only in the case of a first-order reaction. In fact A represents the rate of reaction at infinite temperature. To get rid of this rather undetermined factor, in practical applications the Arrhenius equation is often reparameterized in the reference form:

$$k = k_{ref}\, e^{\frac{-E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)} \tag{3.37}$$

where the index ref refers to an arbitrarily chosen value of T, preferably in the middle of the studied temperature region.

Obviously, E_a relates to the activation enthalpy $\Delta H^{\ddagger}$ and the exact relationship is found as follows. From equation (3.35) it follows that,

$$\ln(k) = \ln\left(\frac{k_B}{h}\right) - \ln\left(\frac{1}{T}\right) + \ln\left(\frac{K^{\ddagger}}{(c^{\theta})^{1-m}}\right) \tag{3.38}$$

hence,

$$\frac{\mathrm{d}\ln(k)}{\mathrm{d}(1/T)} = -T + \frac{\mathrm{d}\ln\left(\dfrac{K^{\ddagger}}{(c^{\theta})^{1-m}}\right)}{\mathrm{d}(1/T)} \tag{3.39}$$

and combining the temperature effect on $K^{\ddagger}$ (the van 't Hoff equation):

$$\frac{\mathrm{d}\ln(k)}{\mathrm{d}(1/T)} = -T - \frac{\Delta H^{\ddagger}}{R} \tag{3.40}$$

From the Arrhenius equation (3.36), it follows that

$$\frac{\mathrm{d}\ln(k)}{\mathrm{d}(1/T)} = -\frac{E_a}{R} \tag{3.41}$$

and consequently combination of equations (3.40) and (3.41) results in

$$E_a = \Delta H^{\ddagger} + RT \tag{3.42}$$

Likewise it can be deduced that the pre-exponential factor A is related to the activation entropy $\Delta S^{\ddagger}$. This makes the factor A much more comprehensible. (The physical meaning of A as such seems to be experienced as somewhat vague, which probably accounts for the fact that the factor A is very often not reported as a result in food science literature. It gives however as much useful information as does E_a.)

Another difference between Arrhenius' and Eyring's expressions is that the temperature T appears in the pre-exponential factor in Eyring's equation (3.35). This has a consequence in the way results are presented and analysed. Very often, Arrhenius' law is presented as a plot of $\ln(k)$ versus $1/T$, which should result in a straight line (if the relationship holds). With Eyring's relationship, $\ln(k/T)$ versus $1/T$ should be plotted. We would like to remark here that it is not a good idea to derive the activation energy parameters from linear regression of $\ln(k)$ or $\ln(k/T)$ versus $1/T$ because of the weighting of data points through logarithmic transformation; rather, non-linear regression should be used.[18] Another remark in this respect is that the two-step procedure of first deriving rate constants at several constant temperatures and then regressing them versus temperature usually results in very wide confidence intervals when only a limited number of temperatures have been studied, as is frequently the case. A better approach is to substitute expressions (3.35), (3.36) and (3.37) directly into the appropriate rate equations and perform a non-linear regression. In this way, all data are used to estimate the activation parameters and an estimate of these parameters of much higher precision is obtained. For representation purposes, it probably remains a good idea to provide Arrhenius' or Eyring's expression in the form of a plot of $\ln(k)$ or $\ln(k/T)$ versus $1/T$ because any deviation of the data from these expressions becomes immediately apparent. In doing so, however, the values of the parameters estimated by non-linear regression should be used to construct the plot. Deviations of Arrhenius' and Eyring's relationship are

indeed possible, but very unlikely. It probably indicates that another reaction influences the one under study, and that problem decomposition is conducted improperly. It is the responsibility of the researcher to check this. In the case that Arrhenius' or Eyring's equations are not applicable (for instance, because an undetected change in mechanism occurs at the higher temperatures), the resulting parameter estimates are worthless. The undetected changes in mechanism have to be incorporated first (see Chapter 2 on problem decomposition) to obtain reliable estimates. So, the first step should always be to check the validity of the laws of Arrhenius/Eyring, and only if they appear to be correct would the next step be the estimation of the activation parameters. Obvious as this may seem, this rule is not always obeyed.

3.2.6   pH effects in kinetic modelling

In many reactions systems pH is of utmost importance. Avoiding interference of changing pH is one of the reasons many experiments are conducted in buffered systems. By the buffering capacity of the applied solutions, the pH remains almost constant at the desired level.

Although the fundamentals of the effects of hydrogen ions on buffering solutions are well understood (as can be taken from almost any textbook on physical chemistry, e.g. Chang[7]), the effects of pH on reactions are still mostly modelled with empirical models. Especially in modelling microbial growth, enzyme activity and quality behaviour in agricultural products, polynomials are frequently used to incorporate the effects of constant but different levels in pH values.[24, 25] The major disadvantage of these models and their estimated parameters is, of course, the fact that it is almost impossible to transfer the values of parameters determined in one experiment to another situation. This limits drastically the application of such models.

The essential action for incorporating the effects of pH on various reaction systems is, again, to find the appropriate reaction mechanism for that system. As with the effect of temperature on the behaviour of reaction rate constants, when the effects of pH are not described by a basic mechanism, one has to attempt to postulate a mechanism, and not just a mathematical fit function.

To incorporate pH into kinetic modelling, one has to realize that pH is defined as $-\log([H^+])$. The concentration of H^+ ions is thus what is important, and all anticipated effects of pH have to be incorporated as H^+ ions. The simplest example is a H^+ catalysed conversion:

$$A + H^+ \xrightarrow{k} B + H^+ \tag{3.43}$$

The constituting differential equations that can be derived from this mechanism are:

$$\frac{d[A]}{dt} = -k\left[H+\right][A] \tag{3.44}$$

As the hydrogen ions are not consumed, the concentration of H^+ can be considered constant, and the analytical solution becomes:

$$[A] = [A]_0 e^{-k[H_0^+]t} \tag{3.45}$$

Exchanging $[H^+]$ for the appropriate pH expression gives:

$$[A] = [A]_0 e^{\left(-k10^{-pH}t\right)} \tag{3.46}$$

So, with this fundamental approach, it becomes not only clear what the effects of pH are, but also by what type of functions: in this mechanism it is effective via a double exponential function.

Another example is when the hydrogen ions are used up in a reaction:

$$A + H^+ \xrightarrow{k} B \tag{3.47}$$

The differential equation for $[A]$ is the same as in the previous example. Now, when the pH is kept constant by buffering action, exactly the same solution as for the previous example emerges: the actual pH does not change. However, in unbuffered systems, the hydrogen ions are used up. By using the mass conservation law, that is $[H^+] - [A]$ is constant at any time, the analytical solution now becomes:

$$[A] \; = \; [A]_0 \frac{[H]_0 - [A]_0}{[H]_0 e^{k([H]_0 - [A]_0)t} - [A]_0} \tag{3.48}$$

Here one can see that a relatively small change in the reaction mechanism studied has a tremendous impact on the behaviour of the reaction components and on deduced equations.

These principles of applying the concentration of hydrogen ions instead of pH directly, has recently been used for describing the combined effects of pH and activating and denaturing temperatures on the activity of the enzyme phytase from various sources.[26] The principles and basic equations had previously been deduced,[5, 6, 7] separately for temperature and for pH, but it was not applied to experimental data hitherto. The combined effects of temperature and pH were validated on seven sets of independently measured data. The fit of the model on the obtained data was remarkable (see Fig. 3.4).

3.2.7   Multiresponse modelling

As indicated above, reactions in foods are usually quite complicated and the use of simple uniresponse kinetics, in which only one response is analysed, can only be an approximation of the underlying mechanism. A very useful technique for studying more complex changes in foods is the multiresponse modelling technique. If it is possible to measure more reactants and/or products simultaneously rather than taking one reactant or one product, one can apply multiresponse modelling. The major advantages of this are that proposed reaction
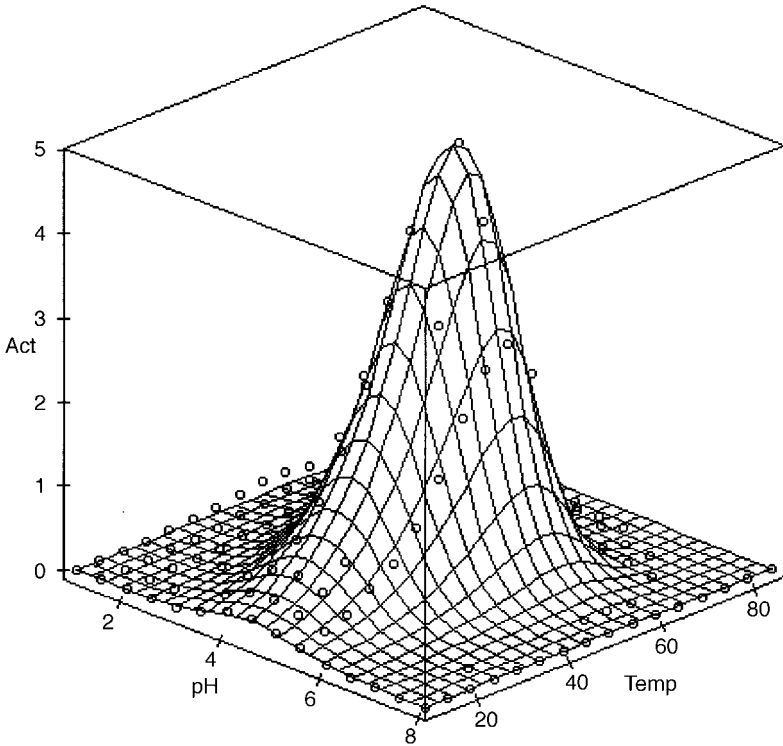
Fig. 3.4   Measured (symbols) and simulated (lines) activity for phytase produced by
E. coli.

models can be tested much more rigorously, and that resulting parameter
estimates are much more precise. The reason for these advantages is that the
information that can be extracted from data is increased considerably. A
disadvantage is that another regression criterion than the familiar least squares
must be used, namely the determinant criterion. This is in itself not more difficult,
but there are only a few software packages that handle this criterion. It is however
very rewarding to apply multiresponse modelling in appropriate cases.[10, 20, 21]

An important aspect for multiresponse modelling is to take variances and
covariances of the various responses into account. To clarify this point, a
hypothetical reaction scheme is discussed first. Suppose three reactions take
place at the same time, and we are able to measure all six components during the
course of the reaction:

$$A + B \xrightarrow{k_1} C + D$$

$$C \xrightarrow{k_2} E$$
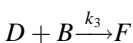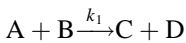
$$D + B \xrightarrow{k_3} F \tag{3.49}$$

with k_i as reaction rate constants. Then the following differential equations can be set up:

$$\frac{d[A]}{dt} = -k_1[A][B]$$

$$\frac{d[B]}{dt} = -k_1[A][B] - k_3[D][B]$$

$$\frac{d[C]}{dt} = k_1[A][B] - k_2[C]$$

$$\frac{d[D]}{dt} = k_1[A][B] - k_3[D][B]$$

$$\frac{d[E]}{dt} = k_2[C]$$

$$\frac{d[F]}{dt} = k_3[D][B] \tag{3.50}$$

These coupled ordinary differential equations (ODEs) can be solved by numerical integration. A well-suited algorithm is, for instance, the Gear routine, especially designed for so-called stiff differential equations (in which the parameters may have largely different values, which is frequently the case for kinetic rate constants).[12] Following this approach we can find a numerical solution describing the evolution of our six compounds over time.

Next, the model (i.e. the numerically integrated rate equations) should be fitted to the experimental data points. The question is how to do that properly. The 'natural' procedure for this would seem to be the method of least squares, for instance to minimize for component A:

$$\sum_{u=1}^{n}(y_A - \hat{y}_A)^2 \tag{3.51}$$

in which u $(1\ldots n)$ is the number of experimental runs, y_A the experimental data points for component A, and $\hat{y}_A$ the predictions of component A by the model (as predicted by numerical integration). In the above example, there are several responses at the same time (the concentrations of components A, B, C, D, E, F at each time interval studied). The question is now whether the best fit criterion in the above example is simply to minimize the combined sum of squares for all responses (like for component A in (equation 3.51). There are several, rather strict, requirements for application of least squares, and these turn out to be very strict, especially in the case of multiresponse modelling. It is not always appreciated by researchers that the fit criterion to be used depends on the experimental error structure: the covariance matrix of the experimental errors, $\mathbf{Cov_{ee}}$, is of importance. For our hypothetical example, this matrix is

$$\mathbf{Cov_{ee}} = \begin{bmatrix} \sigma_{AA} & \sigma_{AB} & \sigma_{AC} & \sigma_{AD} & \sigma_{AE} & \sigma_{AF} \\ \sigma_{BA} & \sigma_{BB} & \sigma_{BC} & \sigma_{BD} & \sigma_{BE} & \sigma_{BF} \\ \sigma_{CA} & \sigma_{CB} & \sigma_{CC} & \sigma_{CD} & \sigma_{CE} & \sigma_{CF} \\ \sigma_{DA} & \sigma_{DB} & \sigma_{DC} & \sigma_{DD} & \sigma_{DE} & \sigma_{DF} \\ \sigma_{EA} & \sigma_{EB} & \sigma_{EC} & \sigma_{ED} & \sigma_{EE} & \sigma_{EF} \\ \sigma_{FA} & \sigma_{FB} & \sigma_{FC} & \sigma_{FD} & \sigma_{FE} & \sigma_{FF} \end{bmatrix} \tag{3.52}$$

The diagonal elements in the matrix $\mathbf{Cov_{ee}}$ represent the variances of each response (i.e., $\sigma_{AA} = \sigma_A{}^2$ and the off-diagonal elements the covariances (i.e., $\sigma_{AB} = \rho\sigma_A\sigma_B$ with ρ the correlation coefficient). The point is that in most cases the covariance matrix of the experimental errors will be unknown. It would be reasonable to assume for multiresponse measurements that measurements in different runs are not correlated, but components measured within one run are expected to be correlated (for instance because several components are determined in one sample). Hence, the covariances $\neq 0$ *within* a run; then the best-fit criterion is not least squares minimization, but minimization of the determinant of the so-called dispersion matrix $\mathbf{C}$ with elements

$$c_{ij} = \sum_{u=1}^{n} [y_u^i - \hat{y}_u^i] \cdot [y_u^j - \hat{y}_u^j] \tag{3.53}$$

in which i, j is the index of responses $(i, j = 1 .. r)$ and u the index of experimental runs $(u = 1 .. n)$. It is perhaps worth noting that the diagonal elements of matrix $\mathbf{C}$ correspond to the sum of squares for each of the responses. The point is that not only the sum of squares for each of the responses is taken into account but also the crossproducts of the responses. This analysis is due to Box and Draper,[17] and since then further developed by several authors. See, for instance, the review article by Stewart *et al.*[15]

If the covariance matrix, equation (3.52), happens to be known, the best-fit criterion is minimization of

$$\sum_{i=1}^{r} \sum_{j=1}^{r} \sigma^{ij} c_{ij} \tag{3.54}$$

in which σ^{ij} are the elements of the inverse of the matrix $\mathbf{Cov_{ee}}$. If, in addition, no correlation exists between responses ($\sigma^{ij} = 0$ for $i \neq j$) and the variances of the responses are known, minimization of the following is appropriate

$$\sum_{i=1}^{r} \sigma^{ii} \sum_{u=1}^{n} [y_u^i - \hat{y}_u^i]^2 \tag{3.55}$$

Equation (3.55) represents the case of weighted least squares. Finally, if σ^{ii} is equal for all responses the minimization criterion is:

$$\sum_{i=1}^{r} \sum_{u=1}^{n} [y_u^i - \hat{y}_u^i]^2 \tag{3.56}$$

and this is actually the least squares criterion for all responses, analogous to equation (3.51) for one component. Coming back now to the question whether or not least squares is the best fit criterion, the answer appears to be: only under the (rather strict) conditions that all variances are the same and that covariances within a run are zero. This is a situation normally not encountered in practice if several responses are measured at the same time. In conclusion, it turns out that in multiresponse modelling the determinant criterion, i.e., minimization of the determinant of matrix (3.53), is the alternative for least squares. In other words, the method of least squares is not well suited for dealing with multiresponse data.[15]

There are two major advantages of multiresponse modelling. The first is that kinetic models can be tested much more rigorously because all the information contained in the data is linked and used. The second advantage is that the resulting estimates of the parameters (once a model is acceptable in terms of goodness of fit and scientific understanding) are much more precise than with uniresponse modelling. Several examples have been given for multiresponse analysis of reactions occurring in foods.[9, 18–21]

3.2.8   The engineering approach

Above, we have described how kinetic equations can be derived from postulated chemical mechanisms. Mathematically, reactions can also be described in terms of the change in concentration c of a component over time t without considering an actual reaction mechanism:

$$-\frac{dc}{dt} = kc^n \tag{3.57}$$

with n the order of the reaction and k the reaction rate constant. This applies to an irreversible reaction of one component (in this case a decomposition, but formation is of course also possible). Integration of equation (3.57) at constant external conditions, gives:

$$c_t^{1-n} = c_0^{1-n} + (n-1)kt \quad (n \neq 1)$$

$$c = c_0 \exp(-kt) \qquad (n = 1) \tag{3.58}$$

with c_0 the initial concentration. There are in fact two types of orders, the first one is found if one determines *initial* rates $-dc/dt$ for various concentrations and then one can determine the order n_c from equation (3.57), which is called the order with respect to concentration. The second possibility is to use equation (3.58) and follow the change in concentration over time, and find the best fit for the order n_t, called the order with respect to time.[3, 18, 22]

When the reaction mechanism reflects the processes occurring in reality, the two orders are necessarily the same. However, when the reaction mechanism applied is a simplification of the reaction process, both orders need not be the same. If $n_t < n_c$ then the reaction rate increases as the reaction moves on (auto-

catalysis); if $n_t > n_c$ the reaction rate decreases as the reaction progresses (auto-inhibition). This indicates that it is useful to determine both types of orders because the comparison of their values should make clear whether or not the mechanism used in the modelling effort changes during the course of the reaction when, for example, autocatalysis or inhibition occurs. If both orders appear to be the same, one can conclude that the reaction under investigation seems to be a simple one and does reflect the occurring processes. However, if both orders show discrepancies, this could be a starting point for further mechanistic investigation.

The value of the order n is usually reported to be between 0 and 3; it need not be an integer value. However, if the order is not integer, it is a clear indication that one should dig deeper into the mechanism at work. It is important to note that equations (3.57) and (3.58) only give a mathematical description, not a mechanistic description of what is going on. In mechanistic terms, a reaction is either monomolecular or bimolecular (very rarely termolecular). An order of 1 or 2 may thus be an indication of a mono- or a bimolecular reaction, but not necessarily. An order that is not 1 or 2 indicates a more complex behaviour, usually because one observes a combination of several reactions. Strictly speaking, the above analysis is only valid for simple irreversible reactions with only one reactant. This is not very realistic for foods. We call this approach the 'engineering approach' because it comes down to mathematical modelling rather than kinetic modelling and is useful only for calculation of conversion rates and the like, which is relevant for engineering purposes, but not for obtaining insight into reaction mechanisms. One should thus realize that it is a crude approximation of the underlying physical or chemical mechanism. It is certainly not valid to deduce mechanistic conclusions and it would be most dangerous to extrapolate beyond the experimental region for which the relation was established. In any case, it may be a starting point for further kinetic analysis. If one wants to dive into more basic reaction mechanisms, one has to take more complex kinetic equations into account, as discussed above.

3.3   Areas of application

Kinetics are very useful in describing changes occurring during food processing and storage. Processing almost always requires a compromise because besides the desired changes, undesired changes will also occur. For instance, during heat sterilization, enzymes and microorganisms are inactivated (desired reactions) but at the same time nutrients such as vitamins and amino acids are degraded (undesired reactions) and undesirable flavour and colour compounds may be formed. Especially during the heating up phase, activation and denaturation of enzymes occur in the intermediate temperature regions.[27–29]

When the effect of temperature on reactions in foods has been established, preferably in the form of the parameters discussed, i.e., activation energy/ enthalpy and activation entropy/pre-exponential factor, the value of the

parameters needs some discussion. Occasionally, there seems to be some misunderstanding regarding interpretation. For instance, if a high activation energy is found, the conclusion is sometimes drawn that the reaction will proceed slowly or with difficulty. This is a wrong conclusion, because the reaction may actually proceed quite fast, namely at high temperature. The point is that a high activation energy indicates a strong temperature dependence, that is to say it will run very slowly at low temperature, but very fast at high temperature. Such differences in activation energy are exploited in processes such as HTST (high temperature short time pasteurization) and UHT (ultra-high temperature processing). These processes are designed based on kinetic knowledge, and result in products with the same shelf-life as their traditional counterparts but with a better quality.

The background of these processes is as follows. Chemical processes leading to quality loss usually have an activation energy in the range of 100 kJ/mol, whereas the inactivation of enzymes and microorganisms has a much higher activation energy of, say, between 200 and 500 kJ/mol. This means that chemical reactions are less temperature sensitive than inactivation of enzymes/microorganisms, in other words by employing a high temperature, microorganisms are inactivated rapidly in a short time, whereas this time is too short to result in appreciable chemical changes.[3] Figure 3.5 illustrates this. Temperatures employed in UHT processing are in the range of 140–150ºC and the times needed are then in the order of a few seconds. Another consequence of importance for foods is that reactions with a relatively low activation energy will proceed at a measurable rate at low temperatures, for instance during storage at low and moderate temperature. In other words, chemical reactions do not stop at low temperature. To prevent spoilage due to chemical reactions in such cases, other measures need to be taken such as drying and induction of glassy states (preventing diffusion of reactants) or by taking away a reactant (e.g. oxygen to prevent chemical oxidation).

When kinetics are applied in food processing, it is necessary to consider additional aspects such as the residence time distribution in equipment, the type
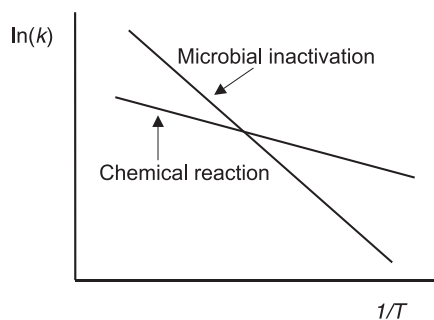

Fig. 3.5 Schematic presentation of the temperature dependence of a chemical reaction and microbial inactivation.

of flow (laminar v. turbulent) and heating-up and cooling-down effects in heat exchangers. These are typical scaling-up engineering problems. These problems are dealt with in Chapters 15 and 16.

When kinetics are applied to foods and living materials, a typical problem is that of compartmentalization, meaning that reactants are physically separated in different compartments or cells, so that they cannot interact. When foods are subsequently processed or damage of cells occurs, the reactants may come together and the reaction proceeds. This can be both desirable and undesirable. In any case it makes the application of kinetics more complicated.

3.4 Pros and cons of kinetic modelling

The advantages of kinetic modelling are manifold.

- The rules for building kinetic models are well rooted in the theories on chemical kinetics and thermodynamics. Consistent application of these rules leads to fundamental and generic models.
- The vast knowledge and information available in the literature and the expertise of experimental researchers can be applied in a way that is consistent with the prevailing and accepted theories.
- With generic models, extrapolations in areas outside the testing area are allowed, provided the processes are governed by the same mechanisms. This also means that model parameters can be validated on separate data sets, obtained for example in favourable laboratory circumstances, and applied in practical situations. Transfer of parameter values is then possible without any difficulty.[30]
- Consistent application of kinetic modelling will avoid, or at least diminish, the burden of scaling-up problems.
- A distinction can be made between kinetic parameters (all rate constants and energies of activation) for fundamental processes and batch parameters (depending on the material in which the reactions take place, i.e. matrix effects). Kinetic parameters are specific for a certain process and therefore have the same value for each repetition or duplication. Batch parameters will depend strongly on the composition of actual batches of agricultural products.[31]

Some disadvantages of kinetic modelling are as follows.

- It is often difficult, if not impossible, to detect and deduce the mechanism at work. Problem decomposition is a major assisting technique to overcome this disadvantage.
- Simplifying the mechanism, without including unnecessary processes and without excluding necessary processes is often very difficult, which is probably the reason that the 'engineering approach' is still widely popular.
- Correct application of kinetic modelling in foods requires insight in chemical kinetics, biochemistry, physics, mathematics and statistics and engineering,

as well as knowledge of the food matrix. It may be difficult to unite all this knowledge in one researcher. It may therefore be better to work in a team with specialists in each of these fields.

3.5   Future trends

One future trend in kinetic modelling is definitely that more complex models will be developed and applied; both developments in software (numerical methods) and in hardware (processor speed) make it possible to construct, analyse and apply very complex models. The bottleneck will, in fact, not be the modelling part, but the experimental validation of models. This remains, of course, a very important issue. The situation calls for far more intense cooperation between specialists in experimental design, experiment conduction, kinetic modelling and statistical analysis. A future trend should be that more attention is paid to the accuracy and precision of experimental determinations, to the precision of the parameters obtained, and to the precision of predictions. In other words, the statistical aspects of modelling and modelling applications should receive more attention. It makes no sense to have very fancy models incapable of making reliable predictions.

Another upcoming trend is molecular modelling with which it becomes possible to predict (bio)chemical changes based on the simulation of molecular behaviour. Although it is not yet possible, one could imagine the possibility of predicting chemical reactivity from the outcome of molecular modelling.

3.6   References

1.   BALL C O and OLSON F C W, *Sterilization in Food Technology: theory, practice and calculations*, New York, McGraw-Hill, 1957.
2.   STUMBO C R, *Thermobacteriology in food processing* (2nd edn), New York, Academic Press, 1972.
3.   VAN BOEKEL M A J S and WALSTRA P, Use of kinetics in studying heat-induced changes in foods. IDF Monograph *Heat-induced changes in milk*. (2nd edn) P. F. Fox (Ed.), Brussels, International Dairy Federation, 1995.
4.   RATKOWSKI D A, *Handbook of Nonlinear Regression Models*, New York, Marcel Dekker, 1990.
5.   WHITAKER J R, *Principles of Enzymology for the Food Sciences* (2nd edn), New York, Marcel Dekker, 1994.
6.   FERSHT A W H, *Enzyme Structure and Mechanism*, New York, Freeman & Co, 1984.
7.   CHANG R, *Physical Chemistry with Applications to Biological Systems*, New York, Macmillan, 1981.
8.   SEGEL I H, In *Enzyme Kinetics. Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems*, New York, John Wiley & Sons, 1993.

9.   VAN BOEKEL M A J S and BRANDS C M J, Heating of sugar-casein solutions: isomerization and Maillard reactions. In: *The Maillard Reaction in Foods and Medicine* (Eds. J. O'Brien, H. E. Nursten, J. Crabbe and J. F. Ames), Cambridge, Royal Society of Chemistry, 1998.

10. VAN BOEKEL M A J S, Modelling of chemical reactions in foods: a multiresponse approach. *Acta Horticulturae* (1998) **476** 149–55.

11. TIJSKENS L M M, RODIS P S, HERTOG M L A T M, KALANTZI U and VAN DIJK C, Kinetics of polygalacturonase activity and firmness of peaches during storage. *J Food Eng* (1998) **35** 111–26.

12. CHESICK J P, Interactive program systems for integration of reaction rate equations. *J Chem Ed* (1988) **65** 599–602.

13. TRNKA O, HARTMAN M and SVOBODA K, An alternative semi-implicit Euler method for the integration of highly stiff nonlinear differential equations. *Computers Chem Engng* (1997) **21** 277–82.

14. ESPOSITO W R and FLOUDAS C A, Global optimization for the parameter estimation of differential-algebraic systems. *Ind Eng Chem Res* (2000) **39** 1291–1310.

15. STEWART W E, CARACOTSIOS M and SØRENSEN J P, Parameter estimation from multiresponse data. *AIChE J* (1992) **38** 641–50.

16. KUZMIC P, Program DYNAFIT for the analysis of enzyme kinetic data: application to HIV proteinase. *Anal Biochem*, 1996 **237** 260–73.

17. BOX G E P and DRAPER N R, The Bayesian estimation of common parameters of several responses. *Biometrika* (1965) **52** 355–65.

18. VAN BOEKEL M A J S, Statistical aspects of kinetic modeling for food science problems. *J Food Sci* (1996) **61** 477–85, 489.

19. VAN BOEKEL M A J S, Kinetic modeling of sugar reactions in heated milk-like systems. *Neth Milk Dairy J* (1996) **50** 245–66.

20. VAN BOEKEL M A J S, Kinetic modelling in food science: a case study on chlorophyll degradation in olives. *J Sci Fd Agric* (2000) **80** 3–9.

21. VAN BOEKEL M A J S, Testing of kinetic models: usefulness of the multiresponse approach as applied to chlorophyll degradation in foods. *Food Res Int* (1999) **32** 261–9.

22. VAN BOEKEL M A J S, Kinetics of heat treatment. *Heat treatment and alternative methods*, Brussels, International Dairy Federation, 1996.

23. TIJSKENS L M M and POLDERDIJK J J, A generic model for keeping quality of vegetable produce during storage and distribution. *Agric Systems* (1996) **51** 431–52.

24. ZWIETERINK M, *Modeling of Microbial Quality*. PhD thesis, Wageningen Agricultural University, The Netherlands, 1993.

25. WIJTZES T, *Modelling the Microbial Quality and Safety of Food*. PhD thesis, Wageningen Agricultural University, The Netherlands, 1996.

26. TIJSKENS L M M, GREINER R, BIEKMAN E S A and KONIETZNY U, Modelling the effect of temperature and pH on the activity of enzymes: the case of phytases. *Biotechnology & Bioengineering* (2000) **72** (3) 323–30.

27. TIJSKENS L M M, RODIS P S, HERTOG M L A T M, WALDRON K W, INGHAM L,

PROXENIA N and VAN DIJK C, Activity of peroxidase during blanching of peaches, carrots and potatoes. *J Food Eng* (1997) **34** 355–70.

28.   TIJSKENS L M M, WALDRON K W, NG A, INGHAM L and VAN DIJK C, The kinetics of pectin methyl esterase in potatoes and carrots during blanching. *J Food Eng* (1997) **34** 371–85.

29.   SCHOKKER E P and VAN BOEKEL M A J S, Heat inactivation of the extracellular proteinase from Pseudomonas fluorescens 22F: inactivation during heating up and cooling periods. *J Dairy Res* (1999) **66** 467–72.

30.   TIJSKENS L M M, HERTOG M L A T M and VAN DIJK C, *Generic Modelling and Practical Applications*. Proceedings COST 915 & COPERNICUS/CIPA Workshop on Food Quality Modelling, B.M. Nicolaï, J. de Baerdemaeker (eds.), Brussels, European Commission, 1998.

31.   TIJSKENS L M M and WILKINSON E C, Behaviour of biological variability in batches during postharvest storage. *Aspects Applied Biology* (1996) **46** 267–8.