THE INFLUENCE OF pH ON THE KINETICS OF ACID HYDROLYSIS OF SUCROSE

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ABSTRACT

Sucrose acid hydrolysis was studied as a potential chemical time-temperature integrator to use under pasteurization conditions. A nonisothermal method was used to determine the kinetic parameters of this reaction at different pH values in the range of 0.8 to 2.5 and covering the range of temperatures from 50 to 90°C. The nonisothermal method was first validated with the classical two-step isothermal method at pH 2.5. Kinetic parameters showed to be highly collinear (correlation of 0.99), but it was concluded that the activation energy can be assumed independent of pH and equal to 99 kJ/mole with the preexponential factor being proportional to the H⁺ concentration. Results are favorable for the future application of this system in the evaluation of pasteurization processes. Since the activation energy was found to be independent of the pH, this system is useful as a TTI for validation of mathematical models, but not so much for monitoring quality factors, except those with an equal activation energy.

INTRODUCTION

The main criterium to establish the efficacy of a thermal process is the microbial safety of the final product (Stumbo 1973) and therefore microbiological methods are commonly used for process assessment (Kessler 1988). In many cases, the heat applied for bacterial inactivation degrades desirable organoleptic and nutritive properties (Stumbo 1973). Studies on the associated destruction of nutrients and quality factors during thermal processing are referred by Lund (1975), Thompson (1982) and others. Mathematical procedures to predict reduction of microorganisms or nutrient destruction in thermal processing were developed (Ball and Olson 1957; Hayakawa and Ball 1969; Teixeira et al. 1969;

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Stumbo 1973), but they all require as input the temperature history of the product. These data were collected for cans and other batch processes using heat penetration tests (Ball and Olson 1957).

Measuring the exact temperature distribution during a continuous thermal process by conventional methods offers obvious problems and therefore research is focusing on the development of alternative on-line methods to indicate the effects of continuous thermal processes on the kinetics of safety and quality factors (Berry et al. 1989; Weng 1991) or simply to validate experimentally temperature profiles in heat exchangers predicted by various mathematical models. For this first purpose, several chemical and biochemical indicators have been proposed in literature. Acid hydrolysis of sucrose was proposed by Lou (1977) and Adams et al. (1984) as a chemical indicator for can sterilization and for UHT processes, respectively.

However, acid hydrolysis of sucrose is also a potential chemical time temperature integrator (TTI) for lower temperature processes, that is, pasteurization. The rate of this reaction might be controlled to the desired sensitivity to pasteurization times and temperatures by selection of the right pH of the sucrose solution. Therefore, the dependence of kinetic parameters of sucrose acid hydrolysis on pH at pasteurization conditions has to be well known.

Hydrolysis of sucrose into its two monosaccharides, commonly called inversion of sucrose, can be catalyzed by acids, bases, salts or enzymes (Vukov 1965; Bender and Brubacher 1973). The mechanism of this reaction is well studied (Bender and Brubacher 1973; Whistler and Daniel 1985). Most research on this subject has been more commercially oriented in two different contexts: (1) the production of liquid sugar by various methods, as reviewed by Marignetti and Mantovani (1979/80), with emphasis on yield and not on the kinetics itself; and (2) the description of hydrolysis of sucrose at very low rates (low temperatures and high pH), to determine conditions of minimum inversion (Honig 1953; Meade 1963). An equation for determining rate constants for various temperatures (20–130°C) and pH (1 to 6.5) was proposed by Vukov (1965), but was not experimentally verified.

To describe the effect of time and temperature on a first order reaction, two parameters are needed (Lund 1975): the rate constant at a reference temperature and the dependence of rate on temperature. In the system under study, the dependence of these parameters on pH also needs to be described. Available kinetic parameters of this reaction are limited and do not cover all ranges of pH and temperature of interest (Table 1). Reported kinetic data do not show a clear relation of these parameters with pH, and frequently published results seem contradictory.

The main objective of this work was the detailed description of the kinetics of sucrose acid hydrolysis at temperatures below 100°C and pH below 2.5 for future use as a TTI in pasteurization processes. Reaction conditions were chosen
<table>
<thead>
<tr>
<th>Reference</th>
<th>pH</th>
<th>$E_a$ (kJ/mol)</th>
<th>$\ln k_0^a$</th>
<th>$\Delta T$ (C)</th>
<th>method</th>
<th>sucrose det.</th>
<th>acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adams et al. (1984)</td>
<td>2.50</td>
<td>106.3</td>
<td>31.78</td>
<td>60-100</td>
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<td>FPD</td>
<td>$H_2SO_4$</td>
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<tr>
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<td>2.5</td>
<td>94.6</td>
<td>27.91</td>
<td>110-140</td>
<td>&quot;</td>
<td>Somogyi-Nelson</td>
<td>$H_2SO_4$</td>
</tr>
<tr>
<td>Sadeghi and Swartzel (1990)</td>
<td>2.5</td>
<td>46.0</td>
<td>15.80</td>
<td>&quot;</td>
<td>EPM</td>
<td>Somogyi-Nelson</td>
<td>$H_2SO_4$</td>
</tr>
<tr>
<td>Lou (1977)$^a$</td>
<td>3.16</td>
<td>94.0</td>
<td>26.27</td>
<td>110-127</td>
<td>isothermal</td>
<td>Glucostat</td>
<td>HCl</td>
</tr>
<tr>
<td>Lou (1977)$^a$</td>
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<td>92.4</td>
<td>25.45</td>
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<td>&quot;</td>
<td>Glucostat</td>
<td>HCl</td>
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<tr>
<td>Lou (1977)$^a$</td>
<td>3.30</td>
<td>99.4</td>
<td>27.16</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Glucostat</td>
<td>HCl</td>
</tr>
<tr>
<td>Rhim et al. (1989b)</td>
<td>3.30</td>
<td>102.3</td>
<td>28.89</td>
<td>70-98</td>
<td>NI - LIT$^d$</td>
<td>enzymatic</td>
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<td>60-90</td>
<td>&quot;</td>
<td>enzymatic</td>
<td>-</td>
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<tr>
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<td>3.40</td>
<td>133.9</td>
<td>36.31</td>
<td>110-127</td>
<td>isothermal</td>
<td>Glucostat</td>
<td>HCl</td>
</tr>
</tbody>
</table>

$^a$ $k_0$ in (min$^{-1}$); $^b$ Data taken from Rhim et al. (1989a); $^c$ equivalent point method; $^d$ nonisothermal with linear increasing temperature
for compatibility with this future application, including the selection of the solvent and the acid catalyst.

THEORETICAL CONSIDERATIONS

Sucrose Hydrolysis

Sucrose hydrolysis gives two reducing monosaccharides according to the following reaction:

\[
\text{Sucrose} + \text{H}_2\text{O} \rightarrow \text{Glucose} + \text{Fructose}
\]  

(1)

This reaction, when catalyzed with acid and at constant pH and temperature, follows first order kinetics (Honig 1953; Meade 1963; Vukov 1965).

\[
- \frac{dS}{dt} = k_T \times S
\]  

(2)

The observed reaction rate constant \(k_T\) increases with decreasing pH with an exponential dependence (Meade 1963; Vukov 1965). Bender and Brubacher (1973) studied the kinetics of acid hydrolysis of sucrose with a mechanistic approach and found the protonation of the sucrose molecule [Eq. (3)] to be the slowest, thus the rate-determining step, with an equilibrium constant \(K_{eq}\) limiting the formation of the intermediate compound [Eq. (4)].

\[
S + \text{H}^+ \rightleftharpoons \text{SH}^+
\]  

(3)

\[
K_{eq} = \frac{\text{SH}^+}{S \times \text{H}^+}
\]  

(4)

The overall rate of disappearance of sucrose is therefore equal to the rate of disappearance of the intermediate rate limiting compound \(\text{SH}^+\) (Bender and Brubacher 1973):

\[
- \frac{dS}{dt} = k_{\text{kin}} \times \text{SH}^+
\]  

(5)
By substitution of Eq. (4) in Eq. (5), the rate of disappearance of sucrose can be related with the sucrose concentration, and the observed rate constant in Eq. (2) is then proportional to the concentration of hydrogen ions by a constant $k^*$ [Eq. (6)].

$$k_T = k_{\text{kin}} \times K_{\text{eq}} \times H^+ = k^* \times H^+$$

(6)

The rate Eq. (2) can therefore be rewritten as Eq. (7), showing that sucrose hydrolysis may be described as a first order reaction both in terms of hydrogen ion and sucrose concentrations:

$$-\frac{dS}{dt} = k^* \times H^+ \times S$$

(7)

The temperature dependence of the observed rate constants follows the Arrhenius equation over a wide range of pH and temperatures (Vukov 1965; Lou 1977) and the activation energy is independent of temperature, as concluded by Ward (1986) from an analysis of data reported by various authors. Therefore:

$$k_T = k_0 \times \exp\left(-\frac{E_a}{RT}\right)$$

(8)

Vukov (1965) stated that $E_a$ is independent of pH and assumed an average value of 108.5 kJ/mole ± 3.0, based on data he gathered in literature. However, more extensive literature review indicates values as different as 46.0 and 133.9 kJ/mole (Table 1).

If the equilibrium constant is assumed to follow an Arrhenius-type temperature dependence (Atkins 1982), and combining Eq. (6) and (8), it can be concluded that the preexponential constant is proportional to $H^+$ and the activation energy is independent of this catalyst:

$$k_0 = k_{0,\text{kin}} \times K_{0,\text{eq}} \times H^+ = k_0^* \times H^+$$

(9)

$$E_a = E_{a,\text{kin}} + E_{a,\text{eq}}$$

(10)

This deduction supports the hypothesis of a pH independent activation energy suggested by Vukov (1965).
Nonisothermal Method

Kinetic studies are directed at the development of a mathematical model describing reaction rate as a function of all relevant variables that are in this case time, temperature and pH (or hydrogen ion concentration). Nonisothermal methods for kinetic parameter determination fit best the aim of this study, as they represent thermal treatments better than the classical isothermal two-step method. Some other advantages of these methods are that they can overcome the problem associated with thermal lag in kinetic studies and simplify the collection of data (Rhim et al. 1989b).

The classical isothermal methods for kinetic parameter determination are two-step methods, where rate constants $k_T$ are obtained at different temperatures from a set of isothermal experiments, according to the following general equation:

$$\frac{dc}{dt} = -k_T c^n$$

(11)

The dependence of the rate constants on temperature is then obtained by fitting the data to the Arrhenius model (Eq. 8), giving the preexponential factor $k_0$ and the activation energy $E_a$.

The same parameters can be obtained from one experiment with changing temperature, where both concentration and temperature are recorded as function of time. This is the basis of nonisothermal methods, which are one-step methods. The rate equation for first-order kinetics ($n = 1$) and the Arrhenius equation combined give the following equation:

$$c_t = c_0 \exp\left\{-k_0 \int_0^t \exp\left(-\frac{E_a}{RT}\right) dt\right\}$$

(12)

A linear temperature increase (approximately) was used. In this case, the temperature evolves gradually with time and the process covers equal temperature ranges in equal intervals of time. In this case there is no analytical solution to the integral in Eq. (12) (Rhim et al. 1989b) and therefore the equation was solved numerically. Integral methods (Hill 1977; Hill and Grieger-Block 1980) were preferred to differential methods, as proposed by Nunes et al. (1991). Nonlinear least squares regression is preferable in this situation (Box et al. 1978). Rate of convergence can be improved and linear dependence of
parameters decreased with a substitution of variables, as proposed by Nelson (1983). A new variable ($\delta$) is introduced, defined as:

$$
\delta = \ln k_0 - \frac{E_a}{R} \beta
$$

(13)

where $\beta$ is the best weight of the temperature profile found for this case. In this situation $\beta$ is:

$$
\beta = \frac{1}{N} \sum_{i=1}^{N} \frac{1}{T_i}
$$

(14)

where $N$ is the number of temperatures $T_i$ recorded during the experiment. The improvements obtained with this transformation have also been observed by Haralampu et al. (1985) for isothermal kinetic studies.

The final equation from which the theoretical concentrations of sucrose were calculated has the following form:

$$
\ln c_t = \ln c_0 - \int_0^t \exp \left[ \delta - \frac{E_a}{R} \frac{1}{T(t)} \beta \right] dt
$$

(15)

The optimization program to obtain the kinetic parameters ($\delta$ and $E_a$) of the reaction (Fig. 1) was written in FORTRAN 77 for running on a personal computer IBM (model 55 SX). The optimization procedure was a nonlinear least squares regression using the Simplex method (Holzman 1980). The objective function was the sum of squares of the residuals between logarithms of predicted and experimental sucrose concentrations, as follows:

$$
o.f. = \Sigma (\ln c_{theo} - \ln c_{exp})^2 \text{ (minimum)}
$$

(16)

The predicted concentrations were calculated at each time by Eq. (15). Restrictions to the Simplex were based on the physical consistency of critical parameters (concentrations and kinetic parameters) and were imposed as suggested by Nedler and Mead (1965). A typical concentration curve is shown in Fig. 2.
FIG. 1. FLOW DIAGRAM OF THE COMPUTER PROGRAM

FIG. 2. EXPERIMENTAL AND PREDICTED SUCROSE CONCENTRATIONS AT pH 1.5 UNDER NONISOTHERMAL CONDITIONS
MATERIALS AND METHODS

Nitric acid was chosen as catalyst based on the following criteria: (1) it was a strong monoprotic acid, (2) it would be inert towards stainless steel of a processing unit at the concentrations used, and (3) it had a good pH stability with time. Tap water was used as solvent, as it would be more convenient for future applications, namely for using in a pilot plant or industrial equipment, where large amounts of solution are necessary. Available tap water had quite constant properties along the year and the reaction in the available tap water gave results with errors in the order of magnitude of demineralized water.

Solutions of nitric acid (Merck) were prepared at pH from 0.8 to 2.5 and standardized by automatic titration against a standard 0.10 M NaOH solution (high performance titration laboratory TitrLab, from Radiometer Copenhagen). Solutions of sucrose (food grade) at 1.0 g/L were prepared from each corresponding acid solution. This concentration was chosen to give final samples for analysis in the range of maximum sensitivity of the analytical method and of the measuring equipment. It was experimentally verified that no significant change in the pH occurred during an experiment.

The sugar solutions were put into 15 duplicate 1.2 × 10 cm stoppered test tubes, which were small enough to avoid a significant thermal lag (maximum verified: 60 s). The tubes were immersed in a thermostatically controlled water bath with reciprocating motion (Shaking Water Bath SW-21C from Julabo). Temperature was increased linearly from 50 to 90°C (Fig. 3) during the time required to achieve 90% reduction of the disaccharide. The sample temperature was monitored with two thermocouples dipped in additional test tubes and was registered at appropriate time intervals. At 15 predetermined time intervals, tubes in duplicate were removed and plunged into cooling water at 5–10°C.

The nonreducing disaccharide concentration was determined by measuring the increase in reducing monosaccharides in the sample, that are the only hydrolysis products. The reducing sugars content was quantified by spectrophotometric dosage at 540 nm (UV/VIS spectrometer UNICAM 8625) by the DNS method as described by Oliveira (1988), which is valid for concentrations between 0.1 and 1 g/L. For each experiment, a calibration curve was made with standard glucose solutions [D(−)-Glucose anhydrous for biochemistry, Merck]. Disaccharide concentration at time t was then obtained by difference of reducing sugar concentration after total hydrolysis and reducing sugar concentration at the same time t.

RESULTS AND DISCUSSION

The acid hydrolysis of sucrose was confirmed to be a first order reaction (see Fig. 4) and the rate constant to follow an Arrhenius dependence on temperature (see Fig. 5) in the range of experimental conditions covered.
FIG. 3. TYPICAL TEMPERATURE HISTORY FOR THE NONISOThermal METHOD (pH 1.5)

FIG. 4. ACID HYDROLYSIS OF SUCROSE AT 70°C (pH 2.5)
Validation of the Nonisothermal Method

The nonisothermal method was validated at pH 2.5, by comparison with results obtained with the classical isothermal method. This pH was chosen because published results at other pH values within the range of temperature/pH of interest to this study were scarce. For this validation 6 isothermal runs were carried out at different temperatures, from 70 to 95°C. The parameters were obtained by the classical two-step approach. In all regressions, the procedure described by Bard (1974), based on the covariance matrix of the estimates, was used for calculating both the correlation factors and the confidence intervals for the parameters. The procedure is only exactly valid for linear regressions, but it has been suggested as a good approximation for nonlinear regressions and is widely used for this situation (Bard 1974; Seber and Wild 1989). The isothermal method showed an increase of the 95% confidence interval of the rate constants with temperature (Fig. 5). The rate constants obtained with the nonisothermal method fall within this confidence interval, thus showing that the two methods provide consistent results.
Dependence of Kinetic Parameters on pH

Using the nonisothermal method the values of $E_a$ and $\ln k_0$ were determined for different pH values from 0.8 to 2.5. The results are plotted on Fig. 6 and 7, respectively. The 95% confidence intervals for each experiment are marked with vertical lines. It can be noted that these intervals have a considerable magnitude. The points show a high scatter as well and a clear relation between the kinetic parameters and pH is not evident. Particularly, it is impossible to conclude that $\ln k_0$ varies linearly with pH, while $E_a$ is independent of pH, as the theory suggests. Due to the significant scattering of the results, it is not possible either to suggest another model that can accurately predict the sucrose concentration variation at all pH values.

It is well known that the $E_a$ and $\ln k_0$ values are highly collinear (Haralampu et al. 1985). In this case this has been confirmed by the calculated statistical correlation between both parameters, which was larger than 0.99. This means that for a given set of data there is a very large number of pairs of $\ln k_0$ and $E_a$ that predict equally well the variation of the rate constant with time. That is, although the plotted values correspond to the minimum residual for a given pH, there is a high number of other $E_a/\ln k_0$ combinations that are statistically similar, with a 95% probability.

![Graph showing the dependence of the activation energy on pH](image)
Therefore, a different analysis is required. Combining Eq. (9) with Eq. (12), a more general relation between concentration and time can be obtained:

\[
\ln q_t = \ln q_0 - H^+ \int_0^t \exp \left[ \ln k_0^* - \frac{E_a}{R T(t)} \right] dt
\]

A nonlinear regression based on this equation was then applied to all experimental data obtained, instead of analyzing each pH set of data separately. The objective function was again the sum of the residuals of the logarithms of sucrose concentration but the parameters considered were \(c_0\), \(\ln k_0^*\) and \(E_a\) (the differences in the computer program flow diagram are represented in bold in Fig. 1). The estimated parameters and corresponding 95% confidence intervals were:

\[
\begin{align*}
  c_0 &= 1.01 \pm 0.01 \text{ g/L} \\
  \ln k_0^* &= 35.6 \pm 0.5 \text{ (}k_0^*\text{ in L/min/mole)} \\
  E_a &= 99 \pm 2 \text{ kJ/mole}
\end{align*}
\]
It should be noted that this procedure does not decrease the collinearity problems between both parameters. Its advantages come from the fact that it is statistically more correct: since a one-time only regression is carried out, there is no propagation of error from one correlation to another.

These values are also shown in Fig. 6 and 7 (thickest line). The 95% confidence intervals are not represented because of their very small magnitude. The activation energy is lower than the one suggested by Vukov (1965) but lies well within the expected range. Figure 7 also shows the dependence of ln $k_0$ on pH using the value of $E_a$ suggested by Vukov (1965): 108.5 kJ/mole. This shows that if a constant $E_a$ value is assumed, then the relationship found for ln $k_0$ as a function of pH is the one predicted theoretically. Obviously, Vukov’s $E_a$ leads to higher residuals, if applied to our data.

Figure 8 shows the predicted variation of concentration with time, at different pH values, using the individual values of ln $k_0$ and $E_a$ obtained at each pH and the $E_a$ and ln $k_0$ values obtained with Eq. (17). It can be observed that the two predicted curves are quite similar.

**FIG. 8. PREDICTED CONCENTRATION PROFILES AT 70°C**

The points were generated with kinetic parameters obtained for each pH. The lines were obtained with $E_a = 99$ kJ/mole and $k_0 = \exp(35.6) \times H^+$ in min$^{-1}$. 
CONCLUSIONS

The isothermal and the nonisothermal method produced consistent results, proving that nonisothermal methods can be used instead of the conventional isothermal ones, having the advantage of reducing time and reagent requirements. Furthermore, in this case the system is tested under conditions similar to the ones it is going to be used for (TTI).

Ea and \( k_v \) values are highly collinear, making the selection of their determination procedure very important. An appropriate analysis allows to conclude that the activation energy of the sucrose acid hydrolysis can be assumed independent of both temperature and pH, while the preexponential factor varies linearly with hydrogen ion concentration, as follows:

\[
k_0 = 2.77 \times 10^{15} \times H^+
\]  

(18)

This behavior has a theoretical support and was validated experimentally by the good fits between such model and experimental results.

Controlling the pH, one can control the rate of the hydrolysis process. For a given temperature a pH can be selected so that after a required time period the variation in the sucrose concentration allows for an accurate determination. Table 2 shows the pH values required to obtain a 90% reduction in the sucrose concentration for different combinations of time/temperature, usual in pasteurization. It can also be noted that the activation energy of this reaction is similar to the activation energy of some quality degradation changes, as Adams et al. (1984) also referred. It is however important to note that this system is only applicable as a TTI for factors exhibiting exactly the same activation energy (Weng 1991). Therefore, its application is far more interesting for experimental verification of mathematical models than for assessing quality changes, where its use is rather restricted, since quality factors can have energy activation as different as 30 and 170 kJ/mole (Lund 1975). It is also very important to note that this reaction should not be used to assess microorganism inactivation, unlike suggested by Lou (1977).

<table>
<thead>
<tr>
<th>Product</th>
<th>Processing conditions</th>
<th>estimated pH for hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>acid products (4.0-4.3)</td>
<td>93.3 C - 5 min</td>
<td>1.74</td>
</tr>
<tr>
<td>acid products (4.3-4.5)</td>
<td>93.3 C - 10 min</td>
<td>2.04</td>
</tr>
<tr>
<td>dairy products</td>
<td>62-65 C - 30 min</td>
<td>1.21 - 1.35</td>
</tr>
<tr>
<td>tomato paste</td>
<td>105 C - 3 min</td>
<td>1.89</td>
</tr>
</tbody>
</table>
ACKNOWLEDGMENTS

The authors acknowledge Junta Nacional de Investigação Científica e Tecnológica (JNICT) and the CEC (AAIR Program) for financial support.

NOMENCLATURE

c Concentration (g/L)
c_0 Initial concentration (g/L)
Ea Activation energy (J/mole)
H^+ Hydrogen ion concentration (mole/L)
k_T Observed kinetic rate constant at temperature T (min^{-1})
k_0 Preexponential factor (min^{-1})
k^* Constant, as defined in Eq. (6) (L/min/mole)
k_0^* Constant, as defined in Eq. (9) (L/min/mole)
k_{kin} Rate constant, as defined in Eq. (5) (min^{-1})
K_{eq} Equilibrium constant, as defined in Eq. (4) (L/mole)
n Order of reaction
n Number of temperatures recorded during an experiment
o.f. Objective function, as defined in Eq. (16)
R Universal gas law constant (= 8.314 J/mole/K)
S Sucrose concentration (mole/L)
SH^+ Protonated sucrose concentration (mole/L)
t Time (min)
T Temperature (K)
TTI Time-temperature integrator
\beta Weight of inverse of temperatures, as defined in Eq. (14) (K^{-1})
\delta Parameter, as defined in Eq. (13)

Subscripts:

exp Experimental
teo Theoretical
t At time t

REFERENCES


