pH effects in foods: Development, Validation and Calibration of a fundamental Model

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SUMMARY

The effects of pH observed in the activity of a number of enzymes from different origins and the degradation of green colour in blanched vegetables, was modelled based on fundamental kinetic principles by considering hydrogen ions as an integral part of the reaction mechanism. Parameters were estimated by non-linear regression analysis with explained parts (R^2_{adj}) was in all cases except for the enzyme lipase, well over 95%, and frequently well over 98%. Applying fundamental models in integral data analysis made it possible to pinpoint aberrations at high and low pH ranges in the discoloration of vegetables, which remained unnoticed using classical analysis procedures. The model on enzyme activity was successfully applied to analyse and to describe a double optimal pH region when iso-enzymes are present in the total pool of active enzymes.

INTRODUCTION

Enzymes play a major role in our daily food, during growth, during processing, during storage, and during eating. The far most important external factor in all processes, including enzymatic ones, is temperature. In certain circumstances, however, pH can also be a significant external factor determining the process kinetics and the process rates. Although the theory on pH behaviour in buffered and unbuffered solutions is, for more then a century, well established and well understood (see previous lecture), the effects of pH are still modelled in a very empirical fashion. Most of the time polynomials of second order or higher are used to model the effect of pH (Zwietering 1993, Wijtzes 1996).

This paper describes a model, which was developed based on simple but very plausible and fundamental considerations and assumptions, for the combined effects of temperature and pH on enzyme activity. The model was calibrated by multiple non-linear regression based on data of phytase activity from seven different origins and on data of lipase and peroxidase activity from hazelnuts. The same fundamental approach was used to described the effects of pH on the degradation of green colour (extraction of Mg^{++} from chlorophyll) in heat treated, frozen and thawed broccoli.

The basic assumption in the enzyme model was a change in enzyme stereo configuration affected by the amount of available H^+-ions. It could be indicated and described by the same model that lipase in hazelnuts occurs in two different active configurations (iso-enzymes). The activity of both iso-enzyme configurations was analysed simultaneously.

In this paper, the developed models and the statistical non-linear regression analysis will be explained and shown in detail. All analyses of the existing data sets with the appropriate model formulations resulted in explained parts (R^2_{adj}) well over 95%.

These results strongly indicate that the approach followed is valid and generally applicable.

MATERIALS AND METHODS

Information on materials and method with respect to produce and measuring methods can be found in Greiner and Konietzny (1999), Tijskens et al.(2001a), Seyhan et al (2001), Gunawan and Barringer (2000) and Tijskens et al (2001b).

The models were developed based on applying the fundamental rules of chemical kinetics based on plausible reaction mechanisms using MapleV, a computer program capable of handling symbolic equations.

Statistical non-linear regression analysis was performed using GENSTAT (Rothamsted, UK).

ENZYME ACTIVITY AND DENATURATION

Effect of Temperature

The denaturation of enzymes and the resulting enzyme activity at higher temperature is already well documented and modelled in literature (Ponne et al 1996, Tijskens et al. 1997-1999, Whitaker 1994 p. 303). The complete process can be simplified as a combination of enzymatic conversion and denaturation by heat:

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This mechanism can be converted into asset of differential equations and solved at constant external conditions like temperature and pH:

$$En = En_0 e^{-k_d t} \tag{2}$$

The apparent activity Act (as measured in experiments) is, as already mentioned, represented by the product of enzyme concentration (En from eq. 3) and the specific activity (k_s) (Godfrey & West 1996, p 491):

$$Act = k_s En = k_s En_0 e^{-k_d t} = Act_0 e^{-k_d t} \tag{3}$$

Effect of pH

As already pointed out (Tijskens and Biekman 2001, Tijskens et al. 2001a and Seyhan et al. 2001), the effect of pH on enzyme activity can be deduced as:

$$Act = \frac{Act_0}{1 + \frac{H^+}{K_{EH}} + \frac{K_w}{K_{EOH}} \cdot \frac{1}{H^+}} \tag{4}$$

For enzyme system exhibiting a double activity region in pH, the activity can be deduced in the same way for every iso-enzyme present. This results in the final model formulation:

$$Act_{overall} = \sum_{i=1}^{n} \left(\frac{Act_{0,i}}{1 + \frac{H^+}{K_{EH,i}} + \frac{K_w}{K_{EOH,i}} \cdot \frac{1}{H^+}} \right) \tag{5}$$

where n is the total number of iso-enzymes present in the system.

Combined effects of temperature and pH

Assuming that the protonation and deprotonation of the active site does not change the heat stability of the three different configurations of one iso-enzyme, equations 3 and 4 can be combined to obtain a full-scale four-dimensional model: the enzyme activity as a function of time, temperature and pH. For one active iso-enzyme this looks as:

$$Act = \frac{Act_{tot,0} e^{-k_d t}}{1 + \frac{H^+}{K_{EH}} + \frac{K_w}{K_{EOH}} \cdot \frac{1}{H^+}} \qquad (6)$$

All reaction rates depend as always on temperature according to Arrhenius law (see Tijskens and Biekman 2001).

Results statistical analysis

The data on phytases from seven different origins obtained at various temperature pH combinations, and on peroxidase and lipase from hazelnuts obtained at one temperature (25 °C), were analysed applying non-linear regression without any data transformation based on eq. 6. In Table 1 the result are shown. The equilibrium constant K_{EH} and K_{EOH} (expressed in the p-notation) were found to be independent of temperature. This means that the protonation and deprotonation of the active site has the same equilibrium values for all temperatures studied. In **Fig. 1** an example is shown for the 3 dimensional behaviour (time, pH, temperature) for the activity of phytase, in **Fig. 2** the activity of lipase iso-enzyme system. The reliability of parameter estimation was extremely high for all phytases and peroxides. Due to a difficult measuring procedure, for lipase the reliability was somewhat lower.

Table 1 Results of statistical analysis of pH and temperature effects on enzyme activity

Estimate	pK_{EH}	pK_{EOH}	$Act_{tot,0}$	E_s	$k_{d,ref}$	E_d	R^2_{adj}	N_{obs}
Dimension	-	-	U/g	kJ/mol	1/min	kJ/mol		
Phytases								
Barley P1	4.67	8.73	22.35	66.38	0.0388	93.41	98.0	240
Barley P2	5.32	7.66	7.14	42.94	0.0012	168.44	97.6	272
E. coli	3.56	8.61	3.27	60.32	0.0024	143.69	98.9	240
Klebsiella terrig.	3.97	8.08	2.96	38.69	0.0001	222.17	98.4	255
Oat	4.64	8.62	40.69	106.82	0.1073	80.79	99.5	195
Rye	4.85	7.60	14.92	40.80	0.0274	55.30	98.2	272
Spelt D21	5.06	7.23	9.43	92.04	0.0382	87.08	97.6	727
T_{ref} °C	40							
Hazelnuts								
POD	5.32	10.22	26.85	-	-	-	96.2	41
Lipase iso 1	4.20	8.54	1.40	-	-	-	89.6	65
Lipase iso 2	6.96	5.47	0.56	-	-	-		
T_{ref} °C	25							

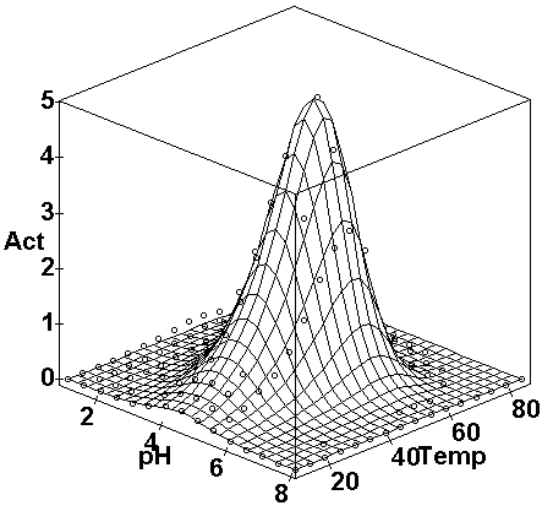

Fig. 1 Measured (symbols) and simulated (lines) activity for phytase produced by E. coli.

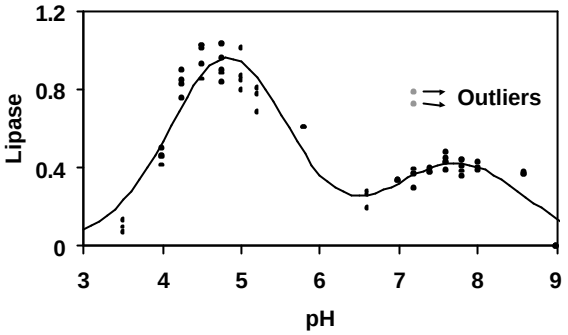

Fig. 2 Activity of Lipase as a function of pH (iso-enzyme system)

COLOUR OF BLANCHED BROCCOLI IN SALAD DRESSINGS

Model development

The same line of fundamental reasoning, bringing the concentration of hydrogen ions into the mechanism, was applied to the degradation of green colour as affected by different pH values to mimic salad dressing. The basic mechanism used is a simple first order reaction, catalysed by H^+ ions (see Tijskens and Biekman 2001:

$$\frac{\partial C}{\partial t} = -k_e \cdot H^+ \cdot C \tag{7}$$

which results in the analytical solution at constant external condition of temperature and pH:

$$C = C_0 \cdot e^{-k_e \cdot 10^{-pH} \cdot t} \tag{8}$$

Applying the derived model to the measured data revealed that at both ends of the pH range applied, this simple model did not adequately describe the observed phenomena. At high pH values, where the concentration of H^+ ions is so low that the apparent rate of reaction is virtually zero, still some degradation of colour was observed. Most probably another pH independent process is going on at higher pH values which is completely masked by the fast pH driven degradation at lower pH values. The model was therefore extended to include also that separate process.

$$\begin{aligned} C + H^+ &\xrightarrow{k_e} P \\ C &\xrightarrow{k_d} P \end{aligned} \tag{9}$$

which can be solve for constant external condition of temperature and pH:

Five different types of acid were used to reach the pre-set pH values, differing in hydrophilicity of the acid tail. To account for this difference, for each acid type a acid factor f_A was estimated separately. This acid factor expresses the difference in extraction

rate constant for the different types of acid. At the same time a simple translation is performed to connect chemical concentrations (C) to the measured CIE L^*, a^*, b^* values

$$ab = ab_{var} \cdot e^{-\left(f_A \cdot k_e \cdot 10^{-pH_{eff}} + k_d\right) \cdot t} + ab_{fix} \quad (10)$$

At the same time some aberration was noted at the very low range of pH values (pH 3 to 3.5). Here the most probable cause is the increasing pH gradient between the low pH value in the buffer solution and the much higher pH values within the product. To counteract this diffusive process, an empirical relation was included in the final model:

$$pH_{eff} = pH_{buf} + f_{pH} \cdot (pH_{int} - pH_{buf})^3 \quad (11)$$

Statistical result Colour degradation

The measured data were, without any data transformation, analysed for all pH values together using non-linear regression based on the combination of Eq 10 and 11. The results are shown in **Table 2**. As can be taken from the explained part the reliability is extremely high (R^2_{adj}=99.5%), certainly when one takes the aberrations at high and low pH values into account, and the broad range in pH values, associated with the very large difference in actual extraction rate for the different pH values. In **Fig. 3** to **Fig. 5** example are given of the behaviour of colour change in blanched broccoli for the entire range in pH

Table 2 Results of statistical non-linear regression analysis

Correction Weight	Both (1/stdev^2)/100	
	Estimate	s.e
ab_{var}	0.78280	0.00964
ab_{fix}	-0.03920	0.00954
k_e	148.70000	4.96975
k_d	4.5260E-06	2.2600E-06
$f_{AAcetic}$	0.91150	0.05952
$f_{ABenzoic}$	5.98491	2.96
$f_{ACitric}$	1	fixed
f_{AMalic}	1.04308	0.04131
$f_{APhtalic}$	1.46687	0.04555
f_{pH}	0.01502	0.00108
N_{obs}	127	
R^2_{adj}	99.5	

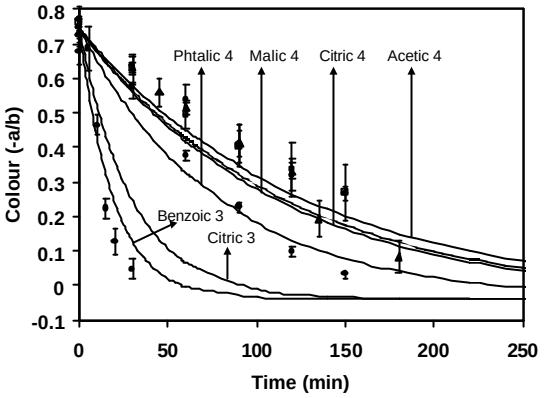

Fig. 3 Colour decay at different pH 3 and 4 (created with different acids). Symbols = measured values, lines = predicted values.

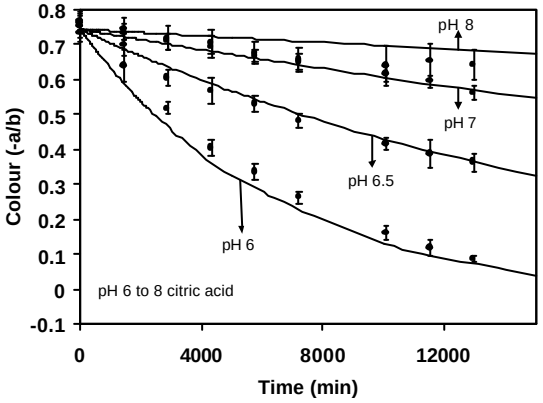

Fig 4 Colour decay at pH 6 to 8 (citric acid). Symbols = measured values, lines = predicted values.

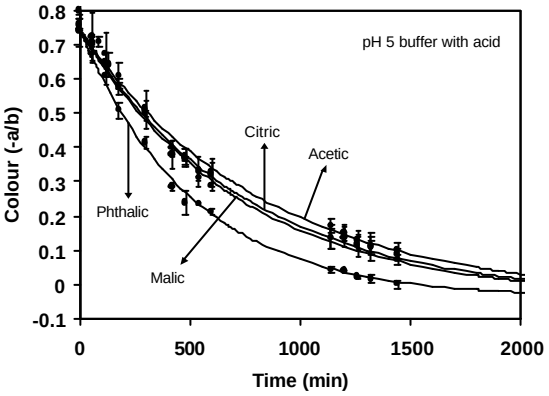

Fig. 5 Colour decay at pH 5 (citric acid). Symbols = measured values, lines = predicted values.

studied. Due to the large difference in rate of colour extraction, the graphs can not be presented in one illustration.

CONCLUSIONS

Effects of pH can easily be modelled on a fundamental basis, provided the concentration of hydrogen ions are used instead of directly pH values. In this approach, hydrogen ions are simply used and described as any other reagent taking part in the mechanism. The model formulations are in complete accordance with the contemporary theories of chemistry and kinetics. All parameters have a distinct and well-defined meaning.

Protonation and deprotonation of active sites of enzymes are independent of temperature. Multiple pH optima in iso-enzyme systems can be described and analysed with the developed model.

Applying fundamental oriented models greatly increases the analysis and interpretation of measured data, and helps in pinpointing aberrations and otherwise unnoticed effects.

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