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Enzyme inactivation kinetics: Coupled effects of temperature and moisture content

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ABSTRACT

Enzymes are often dried for stability reasons and to facilitate handling. However, they are often susceptible to inactivation during drying. It is generally known that temperature and moisture content influence the enzyme inactivation kinetics. However, the coupled effect of both variables on enzyme inactivation over a broad temperature–moisture content range is still not well understood. Therefore, the inactivation of β-galactosidase in maltodextrin matrix is investigated using a newly developed method. An improved kinetic modelling approach is introduced, to predict the inactivation over a large range of temperature–moisture values. The model assumes a two-step inactivation mechanism involving reversible unfolding and irreversible inactivation. The model is able to describe the inactivation kinetics of β-galactosidase accurately, showing the temperature-dependent kinetic transition from reversible unfolding to irreversible inactivation limited. Application of this approach can provide immediate understanding of the effect of processing on enzyme inactivation and indicates the processes' critical points, which offers the possibility for optimisation.

1. Introduction

Many applications have been developed in the chemical, food and biotechnological industry that utilise enzymes to produce or improve products. To facilitate handling and for stability reasons industrial enzymes are often dried. Unfortunately, enzymes are heat sensitive and thus may be inactivated during drying, either partly or completely, depending on the specific drying procedure applied (Chen & Patel, 2008; Sutter et al., 2007; Yoshii, Neoh, Furuta, & Ohkawara, 2008). Enzymes that are susceptible to heat inactivation include glucose oxidase, β-galactosidase, alkaline phosphatase, and lactate hydrogenase (Sanchez & Pilosof, 2006). Generally, it is found that the rate of enzyme inactivation increases with temperature and moisture content (Etzel, Suen, Halverson, & Budijono, 1996; Luyben, Liou, & Bruin, 1982; Meerdink & Van't Riet, 1991; Yamamoto & Sano, 1992). Kinetic modelling of the enzyme inactivation helps to understand how to optimise drying processes with respect to maximum retention of enzyme activity (Perdana, Fox, Schutyser, & Boom, 2011).

One of the major challenges for kinetic modelling of enzyme inactivation during drying is to accurately describe the combined effect of temperature and moisture content on inactivation over a broad range. This is essential, since particles are subjected to this broad range of temperature–moisture content combinations during a single drying process. Luyben et al. (1982) describe an approach to model the kinetics of enzyme inactivation during drying, which was later modified by Yamamoto and Sano (1992). Both references include the combined effect of temperature and moisture content on inactivation. To calibrate kinetic models for a specific enzyme, usually heating experiments are carried out at a constant temperature and constant moisture content. Heat inactivation studies in dilute solutions are very much straightforward. However, collection of accurate inactivation data at lower moisture contents is less straightforward. Several procedures have been developed to investigate the inactivation at low moisture content, for example, in a dedicated inactivation cell (Liou, 1982).

In this paper an improved kinetic modelling approach is introduced to predict enzyme inactivation over a broad range of temperature and moisture values. The approach assumes a two-step enzyme inactivation mechanism that includes a reversible unfolding and an irreversible inactivation step. The major difference between this approach and that of previous work is that it also takes into account the reversible unfolding reaction instead of irreversible inactivation alone. Taking this approach, it is expected that we can describe enzyme inactivation over a large range of temperatures and (low) moisture levels, making it feasible to more accurately describe enzyme inactivation during drying. A disadvantage of a more complex inactivation model is that the number of parameters increases (from 5 to 9). To alleviate this complication, we adopted the conformational stability theory to better describe the reversible unfolding of proteins from a thermodynamic point

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Nomenclature

A	enzyme activity (−)
E_a	activation energy (J mol^{-1})
$\Delta\Delta G$	Gibbs' free energy difference for protein unfolding in a solution with infinite dilution (x_w = 1) and in pure solid form (x_w = 0) (J mol^{-1})
h	Planck's constant (6.626×10^{-34}) (J s^{-1})
$\Delta H^{\ddagger}$	activation enthalpy (J mol^{-1})
$\Delta\Delta H^{\ddagger}$	activation enthalpy difference between unfolding and refolding reaction (J mol^{-1})
I	inactivated enzyme (−)
k	inactivation kinetic constant (s^{-1})
k_B	Boltzmann's constant (1.381×10^{-23}) (J K^{-1})
K_1	reversible unfolding equilibrium constant (mol mol^{-1} or s s^{-1})
m	parameter to describe the effect of moisture content on the conformational stability of protein (−)
N	native enzyme (−)
p	parameter in the Model 1 to describe the effect of moisture content on inactivation kinetic constant (−)
P	uncertainty of the parameters (−)
r	inactivation rate (mol s^{-1})
R	ideal gas constant (8.314) (J mol^{-1} K^{-1})
S	concentration of solids (mol)
$\Delta S^{\ddagger}$	activation entropy (J mol^{-1} K^{-1})
$\Delta\Delta S^{\ddagger}$	activation entropy difference between unfolding and refolding reaction (J mol^{-1} K^{-1})
t	time (s)
T	temperature (K)
U	unfolded enzyme (−)
x	mass fraction (kg kg total^{-1})

Subscript:

0	initial condition
1	first step inactivation: unfolding reaction
−1	first step inactivation: refolding reaction
2	second step inactivation, i.e. irreversible inactivation
crit	critical
I	inactivated enzyme
int	intercept
obs	observed
ref	reference
s	in pure solid form (x_w = 0)
w	in a solution with infinite dilution (x_w = 1)

of view (Tanford, Anfinsen, & Frederic, 1970). Besides the more mechanistic approach in describing the unfolding process the latter also leads to a reduction of the number of parameters in the model (to 8). Finally, the transitional-state theory (Eyring, 1935) is adopted to replace the Arrhenius equation for describing the dependence of inactivation on temperature. The transitional-state theory provides a more mechanistic approach in describing enzyme inactivation as a thermodynamic transition (Cornish-Bowden, 2004).

The kinetic modelling approach introduced here was compared to the previously published model by Luyben et al. (1982) and was evaluated for its accuracy in describing enzyme inactivation as a function of temperature and moisture content. The model by Luyben et al. (1982) is to our knowledge the only published semi-mechanistic model applied to describe enzyme inactivation for a wide range of moisture contents. We used β-galactosidase in a maltodextrin solution as a model system. The enzyme inactivation kinetic constants were collected from heating experiments with temperatures between 55 and 130 °C and with moisture contents between 0.01 and 0.98. Dedicated experimental procedures were used to obtain accurate data at these varying temperatures and moisture contents.

2. Theory

2.1. Enzyme inactivation kinetics

Enzyme inactivation can be described by a reversible unfolding reaction followed by an irreversible reaction. The latter reaction leads to complete inactivation of the enzyme (Apenten & Berthalon, 1994; Jakób et al., 2010; Lumry & Eyring, 1954; Malcolm & Radda, 1970; Schokker & van Boekel, 1997). This can be captured in the following reaction scheme:

$$N \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} U \xrightarrow{k_2} I$$

where N is the native enzyme, U is the unfolded enzyme, and I is the inactivated enzyme.

In the case of β-galactosidase and also for many other enzymes, the reactions involved are usually described by first order kinetics (van Boekel, 2009; Yamamoto & Sano, 1992). The formation rate of the inactivated enzyme, r_I, is thus

$$r_I = \frac{dI}{dt} = k_2 U \tag{1}$$

The unfolded enzyme U is assumed to refold completely upon cooling and convert back to N. By assuming that N and U are in instant equilibrium, the inactivation rate can be described by a first order reaction as a function of $N + U$, which is measured as N after cooling:

$$-\frac{d(N+U)}{dt} = k_{obs}(N+U); \quad N+U+I = N_0 \tag{2}$$

with N_0 is the initial enzyme activity and k_{obs} (observed inactivation rate constant) described as (van Boekel, 2009)

$$k_{obs} = \left(\frac{K_1}{1+K_1}\right)k_2; \quad K_1 = \frac{k_1}{k_{-1}} \tag{3}$$

in which K_1 is the unfolding equilibrium constant, k_1, and k_{-1} are the unfolding and refolding kinetic constants, and k_2 is the irreversible inactivation kinetic constant.

2.2. Model 1

The temperature dependence of enzyme inactivation k_{obs} can be described with the following modified Arrhenius equation (Luyben et al., 1982; Yamamoto & Sano, 1992):

$$k_{obs} = k_{ref}\ \exp\left[-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right] \tag{4}$$

where T is temperature, T_{ref} is reference temperature, k_{ref} is the inactivation rate constant at T_{ref}, E_a is the activation energy, and R is the ideal gas constant.

A lower moisture content has a decreasing effect on the rate of inactivation of enzymes (Luyben et al., 1982; Miyawaki, 2009; van Boekel, 2009; Yamamoto & Sano, 1992). Luyben et al. (1982)