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Thermal inactivation kinetics of β-galactosidase during bread baking

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ABSTRACT

In this study, β-galactosidase was utilized as a model enzyme to investigate the mechanism of enzyme inactivation during bread baking. Thermal inactivation of β-galactosidase was investigated in a wheat flour/water system at varying temperature-moisture content combinations, and in bread during baking at 175 or 205 °C. In the wheat flour/water system, the thermostability of β-galactosidase increased with decreased moisture content, and a kinetic model was accurately fitted to the corresponding inactivation data (R^2 = 0.99). Interestingly, the residual enzyme activity in the bread crust (about 30%) was hundredfold higher than that in the crumb (about 0.3%) after baking, despite the higher temperature in the crust throughout baking. This result suggested that the reduced moisture content in the crust increased the thermostability of the enzyme. Subsequently, the kinetic model reasonably predicted the enzyme inactivation in the crumb using the same parameters derived from the wheat flour/water system. However, the model predicted a lower residual enzyme activity in the crust compared with the experimental result, which indicated that the structure of the crust may influence the enzyme inactivation mechanism during baking. The results reported can provide a quantitative understanding of the thermal inactivation kinetics of enzyme during baking, which is essential to better retain enzymatic activity in bakery products supplemented with heat-sensitive enzymes.

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1. Introduction

Enzymes are naturally present or deliberately added in foods as processing-aids. During processing at elevated temperatures the activity of enzymes is generally affected strongly. For many foods, it is desired to have low residual enzyme activity to stabilize the quality of the product. Examples where high residual enzyme activity detrimentally affects food quality are enzymatic browning in vegetables and fruits, starch degradation in grain products, and enzymatic reactions causing rancidity and off-flavors, etc. (Doblado-Maldonado, Arndt, & Rose, 2013; Kalita, Sarma, & Srivastava, 2017; Lante, Tinello, & Nicoletto, 2016; Luyben, Liou, & Bruin, 1982). In some other cases, one strives to retain high residual activity of the enzyme after processing, for example when the enzyme is used as a food ingredient or a processing-aid to achieve a specific transformation (Guidini, Fischer, Resende, De Cardoso, & Ribeiro, 2011; Ramos & Malcata, 2011; Wu, Dong, Lu, & Li, 2010). To control the degree of enzyme inactivation, especially during thermal processing, it is therefore of importance to have quantitative understanding of the enzyme inactivation kinetics (Illanes, Wilson, & Tomasello, 2000, 2001; Ladero, Ferrero, Vian, & Santos, 2005; Ladero, Santos, & García-Ochoa, 2006).

In this study, the well-characterized enzyme β-galactosidase (β-d-galactoside galactohydrolase EC 3.2.1.23) was selected as the model enzyme. The activity of β-galactosidase is affected by many factors, such as the temperature, moisture content, substrate binding, pH and the presence of salts (Adams, 1991). These effects can be quantitatively described with kinetic models, which can then be used to optimize the residual activity after processing (Ladero et al., 2006). Previous studies showed that the thermal inactivation mechanism of β-galactosidase consists of a reversible unfolding step and a second irreversible reaction step following the widely accepted Lumry-Eyring approach (Ladero et al., 2005; Lumry & Eyring, 1954; Perdana, Fox, Schutyser, & Boom, 2012; van Boekel, 2009).

Several studies investigated the thermal inactivation kinetics of β-galactosidase in dilute solutions, and first order kinetics are generally used to describe the reactions involved in the inactivation of β-galactosidase in solutions (Ladero et al., 2005; Yoshioka, Aso,

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Nomenclature

A	enzyme activity (–)
E_a	activation energy (J mol^{-1})
ΔG	Gibbs' free energy (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})
m	parameter to describe the effect of moisture content on the conformational stability of protein (–)
N	native enzyme (–)
p	parameter to describe the effect of moisture content on inactivation kinetic constant (–)
R	ideal gas constant (8.314) (J mol^{-1} K^{-1})
$\Delta S^{\ddagger}$	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)
Δt	increment of time (s)
T	temperature (K)
U	unfolded enzyme (–)
x	mass fraction (kg kg total^{-1})
n	number of data points (48) (–)

Subscript

0	initial condition
1	unfolding reaction
−1	refolding reaction
2	irreversible inactivation
int	intercept
obs	observed
ref	reference
s	in pure solid form ($x_w = 0$)
w	in a solution with infinite dilution ($x_w = 1$)
g	glass transition

Izutsu, & Kojima, 1994). However, relatively few studies have addressed enzyme inactivation in systems with very low moisture content, which is relevant for processes such as drying, baking or other processes involving changes in moisture content. It is also known that presence of other components such as carbohydrates affects the thermal inactivation of β-galactosidase during drying (Ladero et al., 2006). A mechanistic model was developed to describe the inactivation of β-galactosidase in maltodextrin as a function of temperature and moisture content in relation to optimal spray drying (Perdana et al., 2012), which is however very different from the environment and conditions relevant to bread baking.

The objective of this study is therefore to develop quantitative understanding of the influence of baking on the inactivation of β-galactosidase. First, the inactivation of β-galactosidase in a wheat flour/water mixture is investigated at varying heating times, temperatures and moisture contents. A kinetic model is then fitted to the experimental inactivation data. Subsequently, the inactivation of β-galactosidase during baking is studied experimentally, where the temperature, moisture content and residual enzyme activity in the bread crust and crumb during baking are monitored as function of baking time. Finally, the kinetic model and the experimentally measured temperature and moisture profiles during baking are combined to describe the enzyme inactivation during baking.

2. Materials and methods

2.1. Enzyme solution preparation

β-galactosidase from *Aspergillus oryzae* (⩾8 units/mg solid, Sigma-Aldrich, Germany) was stored at −20 °C and remained fully active throughout the work. The enzyme solution (1% w/w) was prepared by dissolving β-galactosidase in McIlvaine's buffer solution. The enzyme solution was stored in the fridge overnight and filtered with a 0.22 μm filter (Sartorius Stedim Biotech GmbH, Germany) before use. The McIlvaine's buffer solution was prepared by mixing 0.2 M Na_2HPO_4 (Sigma-Aldrich, Germany) and 0.1 M citric acid solutions ($C_6H_8O_7$, Sigma-Aldrich, Germany), and the pH was adjusted to 6.00 ± 0.01.

2.2. Isothermal heating experiments at different moisture contents

Isothermal heating experiments were carried out following two different procedures depending on the moisture content of the flour/water mixtures.

1. For high moisture contents (70%, 80%, 90% w/w), flour suspensions were prepared by mixing wheat flour (C1000®, The Netherlands) into McIlvaine's buffer solution. For heat treatment, 400 μL of flour suspensions was transferred into 2 mL centrifuge tubes (Eppendorf, Germany) and pre-heated to a desired temperature (60 °C–70 °C) in a Thermomixer (Eppendorf, Germany) with a rotation speed of 900 rpm. After preheating, 100 μenzyme solution was added and heated for the designated time. In one centrifuge tube, 100 μL buffer solution instead of enzyme solution was added as blank. After heating, 1500 μL cold buffer (4 °C) was added and the centrifuge tubes were transferred to an ice bath to prevent any further inactivation of β-galactosidase.
2. For the moisture contents 20% and 40% w/w, a different procedure was applied. In this case, a flour paste (40% w/w) was made by mixing wheat flour and the 1% w/w enzyme solution. To obtain 20% w/w moisture content, 0.100 g flour paste (40% w/w) was transferred into a 200 μL centrifuge tube and dried at 25 °C in a vacuum oven to a final weight of 0.075 g. Subsequently, the dough mixtures were heated in a PCR machine (DongSheng®, China) at 65 °C, 70 °C, 75 °C, 80 °C, and 90 °C, respectively, for a certain time. The influence of heating-up time on the experiment can be ignored compared to the total heating time. After heating, the sample was dispersed into 1000 μL cold buffer solution (4 °C) in a 1.5 mL centrifuge tube with two stainless steel balls inside (diameter = 3.0 mm). The suspension was re-suspended in a tissuelyser (JingXin Industrial Development Co., Ltd, China) at 60 Hz for 1 min to extract β-galactosidase from the dried matrix.

2.3. Bread baking experiment

Bread samples were prepared following a no-time bread-baking procedure. In brief, wheat flour (50 g), instant yeast (0.5 g), and 1% w/w enzyme solution (30 mL) were mixed manually, and then the

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