



# Amylolysis of maize mutant starches described with a fractal-like kinetics model



Kamal Kansou<sup>a,\*</sup>, Alain Buléon<sup>a</sup>, Catherine Gérard<sup>b</sup>, Agnès Rolland-Sabaté<sup>a</sup>

<sup>a</sup> INRA – Biopolymères Interactions Assemblages (UR1268), F-44300 Nantes, France

<sup>b</sup> Mondelez France R&D SAS, Mondelez International Company

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## ABSTRACT

Two empirical models, a conventional first-order kinetics and a fractal-like first-order kinetic model were tested for analysing the hydrolysis of 13 wild type, single and double mutants of maize starch by porcine pancreatic  $\alpha$ -amylase (PPA). The major difference between the two models is an additional parameter, the fractal kinetics exponent  $h$ , which enables to characterise a decrease of the reaction rate coefficient over time. The fractal-like kinetic model should be preferred to characterise the amylolysis for 10 mutants out of 13 as sugary-2 and amylose-extender curves exhibit clear reaction rate retardation, unlike normal maize and waxy maize. Analysis of the model parameter values reveals two groups of kinetics for the maize mutants: amylose-extender, normal and waxy on one hand, sugary-2 on the other hand. Possible relations between the parameters of the model and granule composition and structure are discussed.

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## 1. Introduction

Enzymatic hydrolysis of native starch is involved in many biological and industrial processes as, for example, starch metabolism in plants, digestion by mammals, malting, fermentation, glucose syrup or bioethanol productions. Many plants store energy in 1–100  $\mu\text{m}$  insoluble starch granules, which are a semi-crystalline arrangement of two  $\alpha$ -D-glucose polymers built mainly on  $\alpha$  (1  $\rightarrow$  4) linkages: amylose and amylopectin (Buléon, Colonna, Planchot, & Ball, 1998; Pérez & Bertoft, 2010). The major component, amylopectin, exhibits an arborescent branched structure with 5–6%  $\alpha$  (1  $\rightarrow$  6) branching points; it is considered as the support of the crystalline domains in the starch granule (French, 1984; Pérez & Bertoft, 2010; Robin, Mercier, Charbonnière, & Guilbot, 1974). The complex and multi-scale structure of starch granules results from the action of different biosynthetic enzymes, including ADP-glucose pyrophosphorylase, starch synthases, starch branching enzymes, and starch debranching enzymes (Buléon et al., 1998), working in a precise sequential mode (Ball et al., 1996). Thus, modifying biosynthetic pathways yields starches with different granule morphologies, amylose:amylopectin ratios

and amylopectin structures. Starch and its two main constitutive macromolecules are degraded by  $\alpha$ -amylases which are the main enzymes involved in the hydrolysis of  $\alpha$  (1  $\rightarrow$  4) glycosidic bonds (Banks & Greenwood, 1975).

Describing mathematically the enzymatic hydrolysis data of native starch is essential to characterise the kinetic properties of the enzyme–substrate system at work. Empirical and kinetic models are often used for this purpose. They have a simple mathematical expression, a limited number of parameters with physical interpretations, and they fit well the hydrolysis curves.

The enzymatic degradation of native starch is a chemical reaction that occurs in a heterogeneous media where the substrate is solid and the enzyme is in solution; therefore, it might not follow classical reaction kinetics. Given the elaborated hierarchical and multi-scale structure of the starch granule, it is generally expected that the diffusion of the enzyme in the granule is driven and limited by the granule organisation (Oates, 1997). The porous structure of the granule, the insolubility of the amylose chains, and the orderly packed crystalline structures can cause diffusion limitation.

Different quantitative descriptions of such hydrolysis kinetics have been proposed in the literature. A popular approach is to consider the enzymatic reaction as a pseudo-first order kinetics (Al-Rabadi, Gilbert, & Gidley, 2009; Goni, Garcia-Alonso, & Saura-Calixto, 1997; Zhang, Dhital, & Gidley, 2013). The kinetic equation is usually cast in its integrated form:

$$X_t = X_\infty(1 - \exp(-k_e t))$$

\* Corresponding author at: Biopolymères, Interactions, Assemblages (BIA), INRA Nantes, Rue de la Géraudière, BP 71627, 44316 Nantes Cedex 3, France.

Tel.: +33 02 40 67 51 49; fax: +33 02 40 67 50 43.

E-mail address: [kamal.kansou@nantes.inra.fr](mailto:kamal.kansou@nantes.inra.fr) (K. Kansou).

where  $t$  is time,  $X_t$  is the instantaneous hydrolysis extent,  $X_\infty$  is  $X_t$  for  $t \rightarrow \infty$ ,  $k_e$  is the pseudo-first order reaction rate constant. When an observed kinetics can be described by this model, it means that for a fraction of the substrate ( $X_\infty$ ), the reaction can be seen as homogeneous for the enzyme at work, which is a powerful simplification.

Depending on the reaction conditions and the source of the starch, this “Exponential model” sometimes fails to describe correctly the amylolysis progress curves. Such a situation can be observed as soon as the first hour (Edwards, Warren, Milligan, Butterworth, & Ellis, 2014). Then it is generally observed that data would better agree with the sum of two or more fractions of starch with different susceptibilities to the enzyme(s). Kinetic models have been proposed to take into account distinct fractions of starch with different susceptibilities (Dona, Pages, Gilbert, Gaborieau, & Kuchel, 2009; Edwards et al., 2014; Park & Rollings, 1994). Those models implement the idea that granules contain fractions of starch hydrolysed at different rates. However, the definition or the physical grounding of such a distinction is currently disputed (Butterworth et al., 2011; Zhang, Ao, & Hamaker, 2006; Zhang et al., 2013).

In line with the work of Kopelman (1988), heterogeneous chemical reactions can exhibit anomalous kinetics, characterised by a reduction of the reaction rate coefficient with time. Based on theoretical and phenomenological considerations, Kopelman (1988) proposed that, in case of a first-order reaction, the reaction rate coefficient decreases with time, following a power law, such as  $k_t \propto t^{-h}$ , where the exponent  $h$  is the fractal exponent and  $k_t$  is the instantaneous reaction rate coefficient. Fractal-like kinetics are initially the expression of diffusion-limited reactions occurring in fractal spaces (Kopelman, 1988). Fractal-like kinetic models have been applied in particular to simulate the kinetics of cellulose hydrolysis (Väljamäe, Kipper, Pettersson, & Johansson, 2003; Xu & Ding, 2007).

In the case of starch hydrolysis by  $\alpha$ -amylases, several elements could possibly explain the decrease of the reaction rate and then of the enzymes activity during the process. The elaborated structure of native starch granules can limit the adsorption or the diffusion of the enzymes; also, aggregation of hydrolysed  $\alpha$ -glucan chains in the course of the process can form enzyme resistant starch (Lopez-Rubio, Flanagan, Shrestha, Gidley, & Gilbert, 2008). Finally starch-containing fractions of different susceptibilities to the  $\alpha$ -amylase would also give the impression that the reaction rate decreases over time. It is necessary to investigate the relations between any of these factors and the amylolysis kinetics and therefore a need to describe accurately the observed kinetics by means of a minimum set of parameters. A simple empirical model derived from the fractal-like kinetic principle can be used for that purpose.

In this context, the goal of this paper is to assess whether using such a fractal-like kinetics model can improve the description and the analysis of the hydrolysis of native starch by  $\alpha$ -amylases with regard to the Exponential model. For this purpose we used a set of hydrolysis curves measured on 13 starches from mutants of maize with porcine pancreatic  $\alpha$ -amylase (PPA), obtained in a previous work (Gérard, Colonna, Buleon, & Planchot, 2001). These mutants have the same genetic background, but they cover a broad range of amylose content, crystallinity, polymorphic type and granule architecture and exhibit extremely diverse hydrolysis kinetics.

## 2. Models description

### 2.1. Models development

Two empirical views of the enzymatic hydrolysis of starch are considered in this work: a pseudo first-order reaction describing a

conventional kinetics (Exponential model) and a pseudo first-order reaction following fractal-like kinetics (Weibull model).

Assuming that the amylolysis of native starch by PPA can be modelled as a first-order reaction, let us call  $k_t$ , the experimental instantaneous first-order reaction rate coefficient, expressed by the experimental hydrolysis kinetics curves. The Exponential model assumes that  $k_t$  can be modelled by a constant reaction rate,  $k_e$ , such as  $k_t \sim k_e$ . Let  $P_\infty$  be the ultimate concentration of the product, if we overlook the conversion coefficient from starch to reducing sugars, then the product is produced following the mass-action law:

$$\frac{dP}{dt} = k_e(P_\infty - P_t) \quad (1)$$

where  $P_t$  is the concentration of the product at time  $t$ ,  $k_e$  is the first order reaction rate constant of dimension: unit of  $\text{time}^{-1}$ . Integrating Eq. (1) gives the familiar first-order equation, used in particular to fit digestibility curves (Goni et al., 1997):

$$P_t = P_\infty(1 - e^{-k_e t}) \quad (2)$$

If  $X_t$  is the instantaneous extent of hydrolysis ranging from 0 (no hydrolysis) to 1 (complete substrate hydrolysis), such as  $X_t = P_t/S_0$ , then dividing both sides of Eq. (2) by the initial substrate concentration,  $S_0$ , gives the Exponential model:

$$X_t = \frac{P_t}{S_0} = \frac{P_\infty}{S_0}(1 - e^{-k_e t}) = X_\infty(1 - e^{-k_e t}) \quad (3)$$

The Weibull model is the integrated form of a first-order differential rate equation, when the reaction rate coefficient  $k_t$  decreases over time following a power law of the reaction time of exponent  $-h$ , such as:

$$k_t \propto t^{-h} \quad (4)$$

$k_t$  is related to the classical reaction rate constant,  $k_1$ , by  $k_1 \sim k_1 t^{-h}$  ( $k_1$  is  $k_{t=1}$ ) (Kopelman, 1988). Substituting  $k_e$  in the previous first order equation, Eq. (1), gives:

$$\frac{dP}{dt} = k_1 t^{-h}(P_\infty - P_t) \quad \text{where } 0 \leq h \leq 1 \quad (t \geq 1) \quad (5)$$

$h$  is an empirical constant, called fractal exponent, that describes the reaction rate retardation over time (Väljamäe et al., 2003). Considering that the cause of rate retardation is the limitation of reactants diffusion, that in turn is due to the heterogeneity level of the medium, then  $h$  is a measurement of the heterogeneity of the reaction medium (Kopelman, 1988). In homogeneous conditions,  $h=0$ , and the Weibull model becomes the Exponential model; in heterogeneous conditions  $0 < h < 1$ . Eq. (4) and its consequents are approximations not correct at very short time, this is why the model applies for  $t \geq 1$  (Kopelman, 1988). Choosing the unit of time is a matter of choice driven by the data at hand, as discussed in Section 2.2. Integrating Eq. (5) and dividing the result by  $S_0$  gives:

$$X_t = \frac{P_t}{S_0} = \frac{P_\infty}{S_0}(1 - e^{-(k_1/(1-h))t^{1-h}}) = X_\infty(1 - e^{-(k_1/(1-h))t^{1-h}}) \quad (6)$$

Different expressions of this model can be found in the literature; in this study we used the common expression:

$$X_t = X_\infty(1 - e^{-k_w t^{1-h}}) \quad \text{with } k_w = \frac{k_1}{1-h} \quad (7)$$

### 2.2. Interpreting the model parameters and kinetics data preparation

$X_\infty$  is present in the two models (Eqs. (3) and (7)), it represents the value of the hydrolysis extent at  $t \rightarrow \infty$ . Conversely the quantity  $1 - X_\infty$  represents the fraction of starch never hydrolysed, or resistant starch.

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