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Improved methodology for analyzing relations between starch digestion kinetics and molecular structure

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ABSTRACT

A new combined methodology for obtaining relations between starch molecular structure and digestion kinetics of starch-based foods is illustrated using published data on cooked rice. Digestibility data are treated with the logarithm of slopes method, giving region(s) where first-order loss kinetics are applicable; accurate values for the rate parameters are obtained by non-linear least-squares. A new method is developed to find independent structural variables for whole and enzymatically-debranched starches. Chain-length distributions of amylose and amylopectin are fitted with models using biologically-meaningful parameters, including accounting for SEC band broadening. While slower digestion rate usually means higher residual starch, this is not always so; both digestion parameters must be considered separately. The treatment shows that digestion kinetics depend on amylose content and also amylose molecular fine structure: the amount of shorter amylose chains and total amylose molecular size. The new combined data-treatment methodology is applicable to a wide range of food systems.

1. Introduction

Starch is a branched glucose polymer with (1 → 4)-α linear links and (1 → 6)-α branch points, comprising amylopectin (with many short-chain branches) and amylose (with a few long-chain branches – despite what is still sometimes stated, it has been long established (Takeda, Shitaozono, & Hizukuri, 1990) that amylose is not entirely linear). Starch provides about half the food energy for humanity. The rate and location of enzymatic digestion of starch is a significant factor in nutritional value: for humans, more slowly digested starch is desirable, while the reverse holds for animal feed. The digestion of starch is a complex process involving enzyme diffusion towards the substrate (starch), adsorption of enzyme on the starch surface and then the enzyme catalytic event involved. During this process, several factors have been found that could alter each of these steps, including the diffusion of enzyme through starch and grain fragments (Dhital, Shrestha, & Gidley, 2010), the distribution of starch granules, amylose/amylopectin ratio and starch molecular structure, and the effects of non-starch components, including protein and amylose-lipid complexes (Hang, Obert, Gironella, & Burton, 2007; Yu et al., 2016). As such, an accurate estimation and/or relations between starch molecular structure and digestion rate of various starch-containing foods is challenging.

Starch is often classified as RDS, SDS and RS (rapidly and slowly digestible starch, resistant starch) in the Englyst classification system (Englyst, Englyst, Hudson, Cole, & Cummings, 1999), although it is now recommended that an alternative kinetic analysis be adopted (Dhital, Warren, Butterworth, Ellis, & Gidley, 2017; Edwards, Warren, Milligan, Butterworth, & Ellis, 2014), especially to detect any changes in the digestion process. *In vitro* digestion data usually comprise the time evolution of the fraction of digested starch, $C(t)$. Data-treatment methods include a simple first-order kinetic analysis (Goni, Garcia-Alonso, & Saura-Calixto, 1997) and the logarithm of the slope (LOS) plot (Butterworth, Warren, Grassby, Patel, & Ellis, 2012; Edwards et al., 2014; Patel, Day, Butterworth, & Ellis, 2014; Poulsen, Ruiter, Visser, & Lonsmann Iversen, 2003). The LOS method is sensitive to any changes of kinetics during starch hydrolysis, as revealed by changes in slope of the LOS plot, $\ln(dC/dt)$ against t. The LOS method also yields values for the digestion first-order rate coefficient(s) k and fraction of residual starch (starch remaining after an extended digestion period), C_{res}. However, while the LOS method is an excellent means to identify if more than a single simple first-order kinetic process is occurring and to identify the region in which this occurs, it involves taking the numerical derivative of discrete rate data points, making it inherently inaccurate (Yu et al., 2018). An improved treatment avoids this problem by using a

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non-linear least-squares fitting (NLLS) of the integrated form of the rate equation:

$$C(t) = (1 - C_{res})exp(-kt) + C_{res} \quad (1)$$

thus avoiding finding numerical derivatives (Yu et al., 2018). Because of the dominance of first-order kinetics, there may or may not be an advantage to fitting ln $C(t)$; here, we use NLLS on both ln $C(t)$ (denoted "Log") and on $C(t)$ (denoted "Norm"), and choose whichever method gives the better result with regard both to the value of the residual and to visual inspection of the fits.

It was helpfully pointed out by an anonymous reviewer that the amount of starch initially digested was somewhat different for different samples. Were there multiple reaction steps, this would lessen the generality of the conclusions. Fortunately, the LOS analysis indicates that first-order kinetics is followed throughout the whole process, which means that rate parameters are independent of initial starting concentrations.

The rate coefficients describing the digestibility of starch-containing foods often depend on the molecular structure of the starch component (Zhang, Sofyan, & Hamaker, 2008), being dependent on chain-length distributions (CLDs) of individual chains and on the molecular sizes of both amylose and amylopectin.

The best way of determining starch structure-property relations is by reducing the starch molecular structural data to a small number of parameters, and looking for correlations between these parameters and the rate coefficient(s) for the digestion kinetics, using a sufficient range of samples for the results to have statistical validity. Previous applications of this methodology, e.g. (Syahariza, Sar, Tizzotti, Hasjim, & Gilbert, 2013; Syahariza et al., 2014), have however been limited because (1) starch structural parameters were fitted empirically to the data, and (2) the digestion kinetics were treated by the LOS method. Both of these have disadvantages, including the following: (1) conclusions drawn from empirical fitting of structural parameters, such as fractions of chains over arbitrarily chosen ranges of degrees of polymerization (DP), may change if different ranges are chosen; (2) the LOS method involves numerical derivatives by finite difference over discrete experimental points, which is inherently inaccurate.

Rather than fitting the structural data empirically, it is much better to use fittings based on modelling the underlying biosynthesis. This removes potential artifacts, such as arbitrary choice of DP range. A biosynthesis-based model has been established to enable the CLDs of amylopectin to be fitted with a small number of biologically meaningful parameters (Alex C Wu & Gilbert, 2010; Wu, Morell, & Gilbert, 2013); as the application of this model has been discussed extensively in quite a few publications (e.g., see (Tikapunya et al., 2017) for an overview), minimal discussion will be given in the present paper. Very recently, a means of also fitting amylose CLD data with biologically meaningful parameters has been developed (Nada, Zou, Li, & Gilbert, 2017). This latter also partially overcomes the problem of band-broadening (discussed below) which arises in the measurement of amylose CLDs by size-exclusion chromatography (*SEC*, a type of gel permeation chromatography).

The present paper applies the new methods given above to results from an earlier study on the digestion of cooked rice (Syahariza et al., 2013). In addition, a new method is introduced to choose structural variables which are truly independent. The aim of this study is to illustrate the application of the new methodology to investigate the relation between starch molecular structure and the in vitro digestibility. Moreover, it serves to see if the correlations so obtained support the conclusions in the original study: that amylose molecular fine structure, as well as amylose content, affect the digestibility kinetics. It was found that the data used here follow only a single first-order process, and thus some of the power of the LOS method (in identifying more than one kinetic region) is not utilized. However, the main purpose of the present paper is to present a new, unified methodology with two innovations, for which the data used serve sufficiently well. Future applications of this new methodology will of course involve processes that are revealed to have more than one step.

2. Methods for data treatment

The present paper is a refitting of extant data (Syahariza et al., 2013); all details of materials and experimental methods, and the chemical compositions of the rice samples, are to be found in this reference. The data were for 13 different varieties (grown in various locations), as given in Supplementary Information Table S1, which also gives the corresponding abbreviation codes.

2.1. Mathematical fitting of amylopectin CLDs

The best way to obtain the CLDs for amylopectin is fluorophore-assisted carbohydrate electrophoresis (FACE) (Morell, Samuel, & O'Shea, 1998). However, it will be seen in the present study that data for these CLDs obtained using the less accurate CLD data indicate no statistically significant correlation between digestibility kinetic parameters, and amylopectin CLDs, so obtaining more accurate data using FACE is regarded as unnecessary for this particular set of samples. FACE directly yields the number CLD $N_{de}(X)$, which is the number of debranched chains of DP X. SEC with differential refractive index (DRI) detection and calibration using a range of standards and the Mark-Houwink relation yields the SEC weight distribution $w(\log X)$ (Vilaplana & Gilbert, 2010a). The weight and number distributions of debranched starch can be inter-converted (Castro, Ward, Gilbert, & Fitzgerald, 2005) through the relation

$$w(\log X) = X^2 N_{de}(X) \quad (2)$$

The fit to the SEC CLDs in the amylopectin region was implemented with the model of Wu and Gilbert (2010, 2013) using publicly available code (https://sourceforge.net/projects/starchcldfit/?source=directory). The model takes as a starting point sets of the enzymes involved in starch synthesis: the various isoforms of starch synthesis, branching and debranching enzymes. In brief, for a given region, it is assumed that the CLD is dominated largely (but by not entirely) by one or two enzyme sets, these comprising one or two of each of a starch synthase, a branching and a debranching enzyme. The final result is the ratio of activities of the starch synthesis and branching enzyme(s) in enzyme set i, β_i, and the relative amount of the overall CLD produced by that set to that produced by the first set: $h_{1,i}$.

2.2. Mathematical fitting of amylose CLDs

Similar to the fitting of amylopectin CLDs, it is reasonable to assume that the different regions of amylose CLDs (the features of which, as stated, need good SEC separation to be readily apparent) also arise out of the existence of distinct enzyme environments. The nature of these different environments is not yet known; they may involve more than one starch branching enzyme (SBE), or perhaps different loci. However, the mechanistic origin of these is not needed for data fitting and, moreover, the data which can be obtained using the new methodology will doubtless be used in the future to shed light on these events.

Unlike amylopectin, the CLDs of amylose are too long to be accurately analyzed by FACE, which can only analyse chains with DP < 180 (Alex C Wu, Li, & Gilbert, 2014). SEC can handle effectively any DP range, but suffers from some experimental artifacts, especially band broadening (Striegel, Yau, Kirkland, & Bly, 2009), which results in the $w(\log X)$ obtained directly from the DRI detector not being the true shape of the actual amylose CLDs.

The data re-fitting here uses a recent model, which fits amylose structural features and compensates for band broadening (Nada et al., 2017). It is based on an assumption which should be frequently applicable: that the number CLD over a region near a local maximum in the number CLD for amylose can be approximated by a single