

Digestion of starch in a dynamic small intestinal model

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Abstract

Purpose The rate and extent of starch digestion have been linked with important health aspects, such as control of obesity and type-2 diabetes. In vitro techniques are often used to study digestion and simulated nutrient absorption; however, the effect of gut motility is often disregarded. The present work aims at studying fundamentals of starch digestion, e.g. the effect of viscosity on digestibility, taking into account both biochemical and engineering (gut motility) parameters.

Methods New small intestinal model (SIM) that realistically mimics gut motility (segmentation) was used to study digestibility and simulated oligosaccharide bio accessibility of (a) model starch solutions; (b) bread formulations. First, the model was compared with the rigorously mixed stirred tank reactor (STR). Then the effects of enzyme concentration/flow rate, starch concentration, and digesta viscosity (addition of guar gum) were evaluated.

Results Compared to the STR, the SIM showed presence of lag phase when no digestive processes could be detected. The effects of enzyme concentration and flow rate appeared to be marginal in the region of mass transfer limited reactions. Addition of guar gum reduced simulated glucose absorption by up to 45 % in model starch solutions and by

35 % in bread formulations, indicating the importance of chyme rheology on nutrient bioaccessibility.

Conclusions Overall, the work highlights the significance of gut motility in digestive processes and offers a powerful tool in nutritional studies that, additionally to biochemical, considers engineering aspects of digestion. The potential to modulate food digestibility and nutrient bioaccessibility by altering food formulation is indicated.

Keywords Starch digestion · Simulated glucose bioaccessibility · Intestinal motility · Dynamic in vitro models

Introduction

In line with the increasing awareness on diet and health, there is a growing interest in understanding the detailed mechanisms of digestion and nutrient absorption in the gut for health and disease [1–7]. According to the latest report from the World Health Organization (WHO), rates of obesity have more than doubled during the past three decades in virtually every country around the world. With approximately 1.9 billion people being overweight and over 600 million adults identified as clinically obese, the problem is reaching epidemic levels with substantial economical implications (WHO 2015). Obesity has been linked with increased risk for non-communicable diseases including cardiovascular, type-2 diabetes, musculoskeletal conditions, Alzheimer's, sexual dysfunction [4, 8–11]. In the UK, the relevant cost to the NHS has been estimated to reach £49.9 billion by 2050 [12, 13].

The majority (80 %) of nutrient absorption occurs in the small intestine, a tubular part of the digestive tract, where chyme enters from the stomach and it is further broken

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down to nutrients, such as glucose, accessible for absorption [14]. Evidence exists that food formulation and microstructure play an important role in digestive processes and postprandial body response, including increase in blood glucose levels [15–22]. An important glycemic carbohydrate component, responsible for a 60–70 % of available carbohydrate in diet, is starch [23, 24]. Starch digestion rate and extent have been directly correlated with postprandial glucose and insulin responses *in vivo* and *in vitro* [2, 23, 25–28]. Starch digestibility is reported to depend on a number of factors such as starch variety, crystallinity, chemical modification, starch structure, food formulation, processing [29–34].

Both *in vivo* and *in vitro* methods have been used in the literature to estimate the effect of form and structure of starches on rates of digestion [21, 32, 33, 35–42]. Because of the expense of *in vivo* physiological assays for the assessment of starch digestibility in humans, *in vitro* methods are desired. These *in vitro* tests employed to quantify digestive processes and simulated nutrient absorption, however, typically disregard the effect of gut motility due to the nature of the investigations and the desired outcome [21, 26, 29, 30, 32, 33, 40–42]. Such ‘batch’, or biochemical, models focus on studying the release and bioaccessibility of active molecules by using a series of vessels, each mimicking digestive conditions (pH, temperature, enzymes) at different parts of the digestive tract [43–45]. On the other hand, dynamic models take into account temporal features of digestion, such as gut motility and fluid motion [46, 47]. The importance of dynamic models has long been acknowledged [48], and a number of models have since been developed to mimic oral [49–53], gastric [54–59] or intestinal [46, 60] digestion, or a combination of those [45, 61–66]. Such models have been used to evaluate the bioavailability of active components [67, 68], stability of antioxidants such as xanthophylls [69] and micronutrients such as iron, folic acid and ferulic acid [70], the effect of allergens in foods [71], release of nutrients or bioactive components [58], performance of functional foods and survival of probiotics [56, 72, 73]. Due to the complexity of food systems, generally multiphase materials, and the complexity and variability of the human digestive system, *in vitro* modelling of digestion is still a challenging research area [44, 45, 74, 75].

Intestinal motility *in vivo* is responsible for mixing and transfer of chyme, enzymes, and other secretions through the tubular intestines. The mixing mechanism is facilitated by gut wall contractions that result in circular constrictions of the tube [14]. These constrictions either divide the intestinal lumen into a number of segments that enhance intestinal mixing (segmentation movements) or enable aboral propagation of the food digesta (peristaltic waves) [14]. Segmentation movements occur at alternating locations

in cycles by gut wall contractions and relaxations, thus moving the intestinal material orally and aborally without net movement. The maximum cycle frequency has been reported at 12 cycles per minute [14].

The main objective of this work is to understand the physicochemical phenomena occurring during starch digestion by using a dynamic *in vitro* small intestine model (SIM). The model simulates the characteristic peristaltic and segmentation movements found in the small intestine *in vivo*, with representative physiological features, volumes and flow rates using the same model, Tharakan et al. [60] have observed up to 30 % reduction in simulated absorption of riboflavin on addition of guar gum. However, in their experiments the effect of enzymatic hydrolysis was not considered. In the present work, the effects of motility, starch and enzyme concentrations, enzyme flow rates, and chyme viscosity (addition of guar gum) on starch digestibility (rates and extent) and associated simulated glucose bioaccessibility are presented. Experiments with a commercial food (white bread) are also shown. Results indicate the importance of chyme viscosity on digestibility as a means to control mass transfer in the small intestine. Overall, this contribution provides an insight of digestive processes in the small intestine combining a nutritional with an engineering approach and highlights the importance of motility and mass transfer in nutrient bioaccessibility for the design of *in vitro* models with improved features. The model offers the benefit of combining biological features of digestion, such as pH and enzymatic secretions, with engineering characteristics. Obtained data can be used to estimate the glycemic index and glycemic load of a variety of foods, correlating food structure with postprandial blood glucose level peaks. Ongoing work aims at correlating current *in vitro* experimental data with *in vivo* results using the same food models. It is thought that in the future, a successful *in vitro* outcome will aid prioritising and strategising clinical studies for nutritional claims as well as for the design of structured foods with improved health benefits.

Materials and methods

All chemicals were purchased from Sigma Aldrich UK, and all experiments were performed at room temperature and in triplicates, unless otherwise stated. Concentration % refers to w/v throughout the text, unless otherwise stated.

Starch solution preparation

Cornstarch (S4126) was dissolved in distilled/deionised water (1.0–3.0 %) and was gelatinized for 30 min in a boiling water bath with intermitted mixing. Samples were left to cool to room temperature and were used within 2–5 h

from preparation. No retrogradation was evident during this storage duration.

Enzyme solution preparation

Pancreatic α -amylase (EC 3.2.1.1. A3176 Type VI-B, ≥ 30 units/mg solid) and amyloglucosidase (EC 3.2.1.3. A7095, ≥ 300 U/mL, aqueous solution) were dissolved in pancreatic mix solution (PMS, 146 mM NaCl, 4.8 mM KCl, 2.6 mM CaCl₂, 0.66 mM MgCl₂, 4.1 μ M ZnSO₄·7H₂O in distilled/deionised water). Selected concentrations are based on reported values by the Institute of Food Research, UK [56]. Enzymes solutions were freshly utilised. Enzyme concentrations are noted in Units of enzyme per mL of chyme (U/mL_{chyme}).

Amyloglucosidase (AMG) from *A. niger* was used to convert maltose and maltodextrins to glucose in the lumen side of the SIM, mimicking hydrolysis occurring by enzymes present in the intestinal wall. It is noted that although AMG may have synergistic or antagonistic actions with α -amylase, which may interfere with data interpretation [76], all experiments were conducted using exactly the same protocol thus reducing the effect of such discrepancies. AMG from *Aspergillus or Rhizopus* have been extensively used in mechanism of digestion in vitro to complete hydrolysis of carbohydrates to glucose [26]. Commercial pancreatin was not used in this work as the amylase activity in unknown, and only starch was used as food model.

Small intestinal model (SIM)

The SIM has been developed at the School of Chemical Engineering, University of Birmingham UK, and has been described in detail elsewhere [60]. The model (schematic of Fig. 1) functions as a concentric mass transfer exchanger

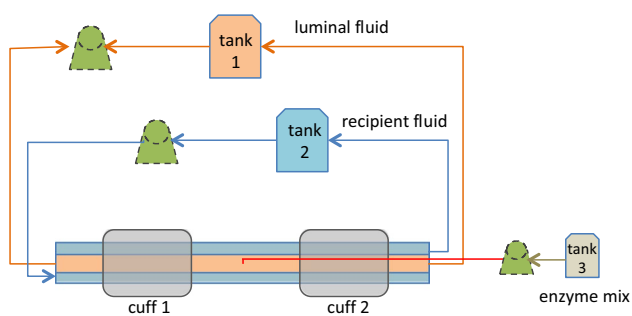


Fig. 1 Schematic of the experimental SIM: food recirculates through tank 1 in the luminal side and recipient fluid (initially water) recirculates through tank 2. Segmentation is induced with the aid of two rubber cuffs with controlled inflation/deflation patterns. Enzyme solution is added at a controlled flow rate in the middle of the luminal side. Starch hydrolysis and simulated glucose absorption are determined from samples obtained from tanks 1 and 2, respectively

composed of an inner semi-permeable RC membrane (Spectra/Por 7[®], MWCO 8 kDa, 32 mm diameter, Medicell International Ltd., London, UK) and an outer non-active, impermeable silicone tube (Flexible Hose Supplies, UK, 50 mm inner diameter, 3 mm thickness). Enzyme solution is fed into the membrane using a polypropylene tube (3 mm diameter, shown in red in Fig. 1) at controlled flow rates (peristaltic pump). Segmentation is simulated by squeezing the membrane using two pneumatically controlled, inflatable cuffs. Alternating cycles of 2-s inflation–2-s deflation–2-s delay were performed, marking a total of 10 cycles per minute, which is within the reported range of maximum 12 cycles per minute occurring in vivo [14]. Recirculation of the luminal and recipient fluids is facilitated by peristaltic pumps. Sample collection is possible from both the luminal and recipient sides (tank 1 and tank 2 in Fig. 1) to study starch hydrolysis and simulated glucose absorption, respectively.

The aim of the SIM is to provide a simple, easy to use and easy to interpret intestinal model that considers the effect of mass transfer, alongside with physiological conditions, in small intestinal digestion and simulated absorption. As such, some simplifications have been adopted. Absorption in the SIM occurs only by diffusion through the porous membrane rather than taking into account the complex in vivo absorption mechanisms. In addition, the model does not take into consideration the effect of the villi, such as local mixing induced by the movement of the villi protrusions and increase in the gut wall surface area, for simplicity. Surface area available for absorption in the present configuration is 0.05 m².

Mixing experiments

The effect of rigorous mixing (baffled stirred tank reactor with Rushton turbine impeller, STR) and segmentation movements (SIM) on starch (1.0 %) hydrolysis was studied (see schematic of Fig. 2). In the STR, impeller speed, temperature, and pH were kept constant at 300 \pm 50 rpm, 25 \pm 2 $^{\circ}$ C and 6.50 \pm 0.05, respectively, and sample volume was 250 mL. Sample volume in the SIM was 600 mL. Enzyme solution (25 mL for the STR and 60 mL for the SIM) was freshly prepared by dissolving the α -amylase (5 U/mL_{chyme}) and amyloglucosidase (0.5 U/mL_{chyme}) into PMS for 10 min at room temperature. Enzyme solution was fed (\sim 3.0 mL/min) into the SIM at approximately 5.0 cm distally to the first cuff (Fig. 1) and into the STR at 3.0 cm from the base. Chyme recirculation was restricted by closing the two ends of the membrane.

Samples were collected from the distal end of the SIM's lumen and the centre of the STR at time zero (prior to enzyme injection) and at time intervals up to 90 min.

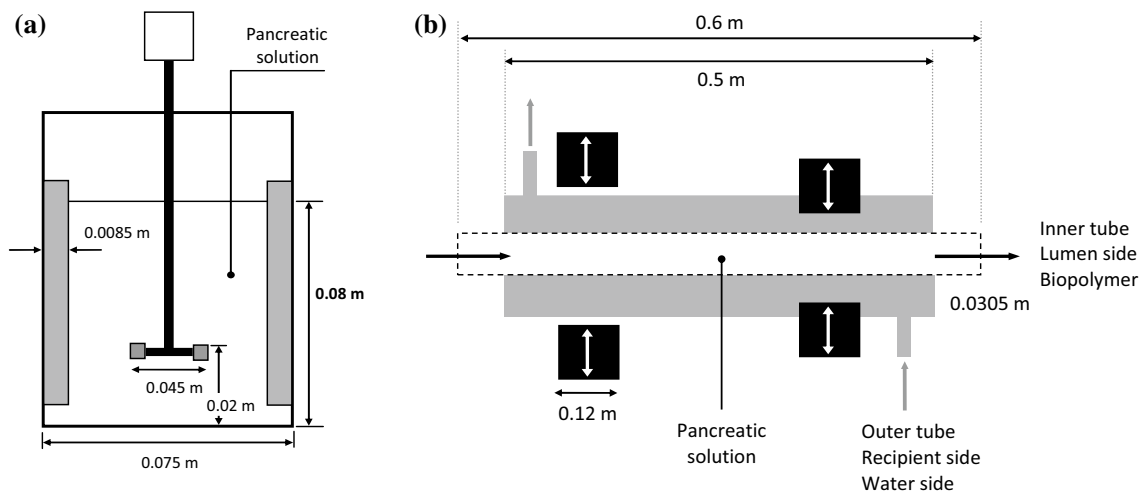


Fig. 2 Models used for the mixing experiment, **a** stirred tank reactor (STR) and **b** small intestine model (SIM)

Table 1 Investigated experimental conditions for starch hydrolysis in the SIM

	Enzyme flow rate (mL/min)	α -Amylase conc. (U/mL _{chyme})	Starch conc. (%w/v)	Guar gum conc. (%w/v)
1	1.5; 3.0; 4.5	10	1.0	–
2	3.0	5; 10; 15; 25	1.0	–
3	3.0	15	0.5; 1.0; 2.0; 3.0	–
4	3.0	15	1.0	0.0; 0.25; 0.50; 0.75

Samples were treated with the DNS method (see “[Sample analysis](#)” section) for determination of reducing sugars.

Effect of enzyme flow rate, enzyme concentration, starch concentration and chyme viscosity

A series of experiments were conducted to study the effect of (1) enzyme solution flow rate (1.5–4.5 mL/min); (2) α -amylase concentration (5–25 U/mL_{chyme}); (3) starch concentration (0.5–3.0 %); (4) system’s viscosity (addition of 0–0.75 % guar gum in the starch solution) on simulated digestibility and nutrient bioaccessibility. Amyloglucosidase concentration was kept constant at 3 U/mL_{chyme}. Table 1 summarises the relevant experimental conditions. Total α -amylase increase with time for experiments 1 and 2 of Table 1 is shown in Fig. 3a, b, respectively. Selected flow rates fall within the range reported by [77] (4.13 ± 0.88 mL/min). Maximum concentration of α -amylase (25 U/mL_{chyme}) was selected according to IFR [56]. Digestion was performed at room temperature in 600 mL chyme consisting of 550 mL pregelatinised starch and 50 mL 0.1 M Bis-Tris buffer (pH 6.5 ± 0.2). Starch hydrolysis was monitored by sampling the lumen, whereas simulated glucose absorption was monitored by sampling the recipient side of the SIM. Samples were treated with

the DNS method (“[Sample analysis](#)” section). Chyme was recirculated along the SIM by a peristaltic pump during 2 h. Experiments with different enzyme concentration/flow rate and starch concentration were conducted in duplicates. Experiments at different guar gum concentrations were conducted in triplicates.

The percentage of starch transformed during in vitro digestion was estimated as follows; the molecular weight of the starch monomer ($C_6H_{10}O_5$)_x is 162 g/mol; therefore, the maximum concentration of total starch in 600 mL of 1.0 % (w/v) starch digesta is 37 mM. This value was used as initial starch concentration. The maximum digested starch measured in the experiment was then used as final starch concentration. For example, since the maximum digested starch concentration achieved in Fig. 5a was ~31.4 mM, the percentage of starch digested after 2 h was estimated at approximately 85 %.

Bread experiments

Fifty grams of white bread with and without 0.5 % guar gum was digested in the SIM, and samples from the recipient side were collected to estimate simulated glucose absorption.

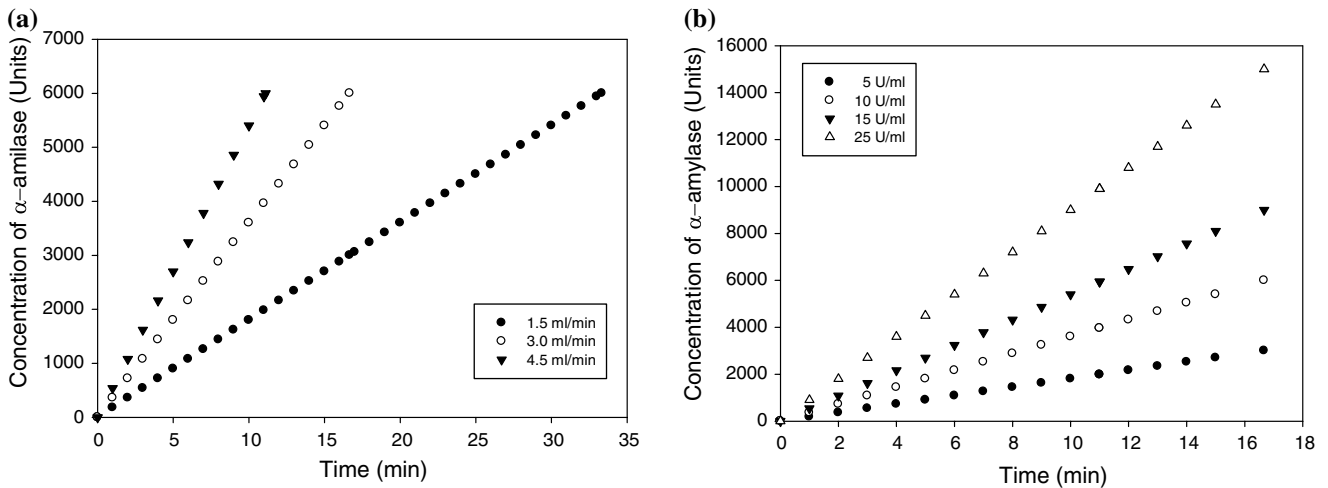


Fig. 3 α -Amylase increase in the luminal side of the SIM for experimental conditions 1 (a) and 2 (b), as shown in Table 1

Sample analysis

Collected samples from the luminal and recipient sides were analysed for reducing sugars using the 3,5-dinitrosalicylic acid (DNS) method [46, 78]. One millilitre sample was mixed with 1 mL DNS reagent (0.1 % dinitrosalicylic acid; 30 % w/w potassium sodium tartrate; 0.4 M NaOH) and immediately placed into boiling water for 5 min to inactivate the enzymes and allow for the redox reaction to take place. Absorption at 540 nm was then measured at room temperature.

Results

Mixing experiments

Figure 4 shows the hydrolysis curves (increase in reducing sugars concentrations during starch hydrolysis) from samples collected from the STR and the SIM. Initial fast apparent starch hydrolysis (44.8 $\mu\text{M/s}$) was observed in the STR, followed by a decreasing rate, until an equilibrium plateau was reached at approximately 20 min, with maximum glucose concentration of 31 mM. Unlike the STR, the SIM showed a sigmoidal starch hydrolysis profile, featuring an initial 10-min “lag phase”, when practically no hydrolysis was observed. Following the lag time, kinetics of starch hydrolysis in the SIM showed the same trend as those in the STR, with a rapid rate (24 $\mu\text{M/s}$, at 10–30 min), a subsequent decreasing rate, and final equilibrium glucose concentration (at 31 mM), reached after 40 min of digestion.

Results were further analysed to quantify the kinetic constants k for the STR and SIM assuming first-order reaction kinetics (Eq. 1), by plotting value of $\ln \frac{[S]}{[S]_0}$ with time and calculating the slope, as seen in Eq. 2 (integrated

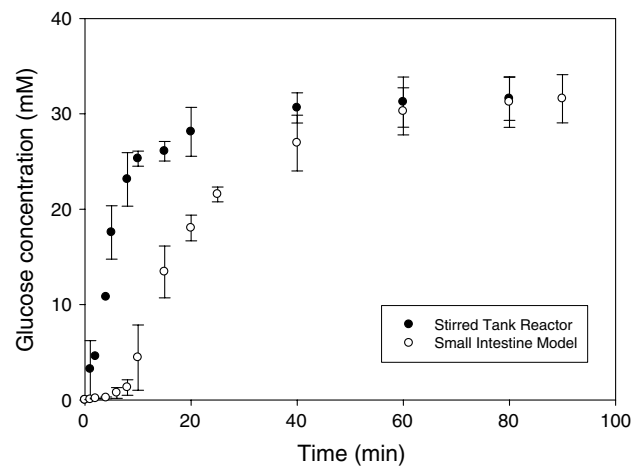


Fig. 4 Starch hydrolysis in the stirred tank reactor (STR) and small intestine model (SIM)

from Eq. 1). Starch concentrations were estimated using data from Fig. 4. It is noted that whereas excellent agreement was found between Eq. 2 and hydrolysis in the STR ($R^2 = 0.94$), results from the SIM indicated some discrepancies ($R^2 < 0.9$). This is probably due to the presence of the lag phase (Fig. 4), which induces deviations from Eq. 2 at initial stages of SIM hydrolysis. The reaction kinetic constants for the STR and the SIM are summarised in Table 2.

$$\frac{d[S]}{dt} = -k[S] \tag{1}$$

$$\ln \frac{[S]}{[S]_0} = -kt \tag{2}$$

S is the concentration of starch at time t , S_0 is the concentration of starch (mM) at $t = 0$, k is the kinetic constant (min^{-1}), and t is time (min) [79].

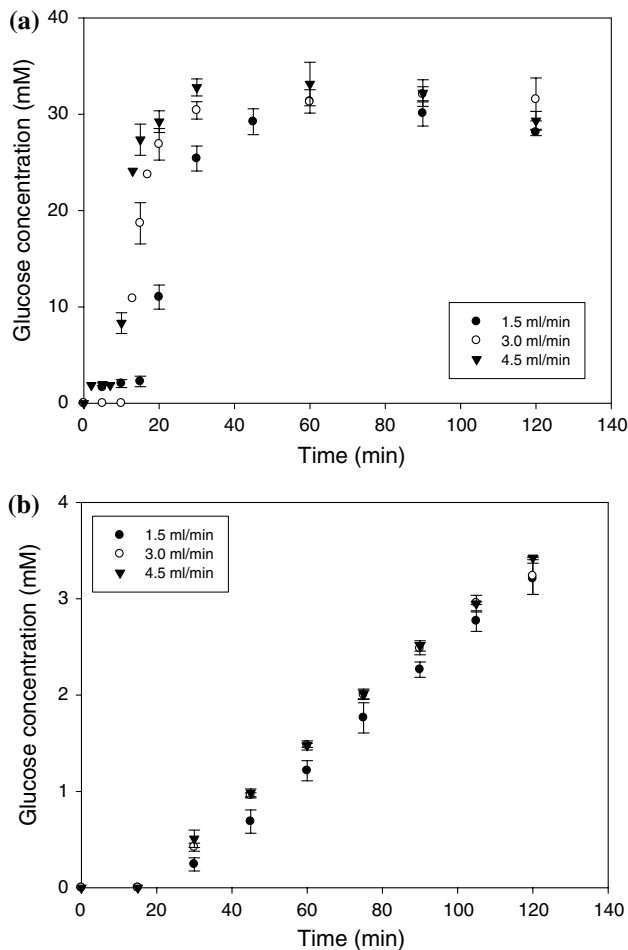


Fig. 5 Starch digestion in the lumen (a) and glucose absorption in the recipient side (b) of the SIM at varying enzymatic flow rates (1.5, 3.0 and 4.5 mL/min). Starch and α -amylase concentration: 1.0 % (w/v) and 10 U/mL_{chyme}

Effect of flow rate of the enzyme solution

Figure 5 shows results of starch digestion in the SIM at different flow rates of the pancreatic solution (1.5, 3.0, and 4.5 mL/min), all other parameters being constant (experimental conditions 1 in Table 1). It is reminded that although enzymes are added at different rates (Fig. 3a), the final enzyme concentrations are the same for all investigated flow rates (α -amylase 10 U/mL_{chyme} amyloglucosidase 3 U/mL_{chyme}).

Figure 5a indicates overall faster starch hydrolysis in the luminal side on increasing enzyme flow rates. Starch was hydrolysed to the extent of 85 %, and maximum glucose concentration (31 mM) was reached after 60, 30 and 30 min for 1.5, 3.0 and 4.5 mL/min, respectively. Interestingly, while increasing flow rate from 1.5 to 3.0 mL/min had a significant effect on both the rate of starch hydrolysis

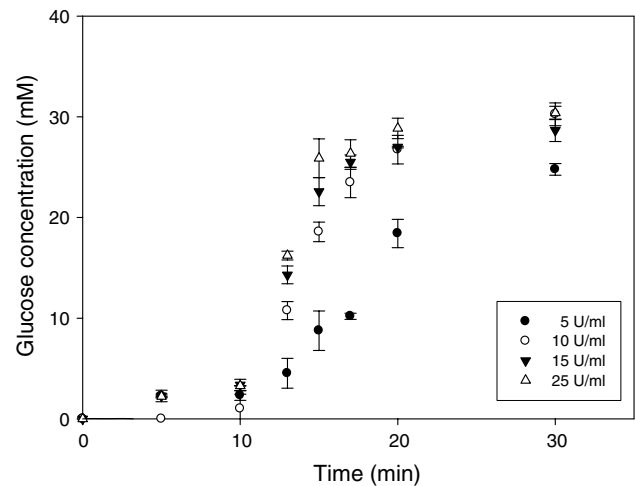


Fig. 6 Starch digestion in the SIM for various amylase activities (5–25 U/mL_{chyme})

and time to reach saturation, further increase to 4.5 mL/min only marginally affected simulated starch digestion.

In agreement with the results of starch hydrolysis, Fig. 5b indicates no significant variation in glucose absorption (samples collected from the recipient side of the SIM) when the enzymatic flow rate increased from 3.0 to 4.5 mL/min. No glucose was detected during the first twenty minutes of digestion (lag phase). Following the lag phase, glucose increased linearly over time reaching a concentration up to 3.5 mM, which represents 10 % of the glucose generated in the lumen of the SIM. As no significant difference was observed in digestion and absorption process between 3.0 and 4.5 mL/min, the flow rate of 3.0 mL/min was selected for the remaining experiments.

Effect of enzyme concentration

The sensitivity of the measurements on enzyme concentration was studied by measuring starch digestion kinetics at different α -amylase activities (Fig. 6), all other parameters being constant (experimental conditions 2 in Table 1). Results shown in Fig. 6 indicate reduced rate of starch hydrolysis at low enzyme concentrations (5 and 10 U/mL_{chyme}). It appears, however, that at 15 U/mL_{chyme} the apparent rate of reaction was not limited by the amount of α -amylase added, and any further increase in enzyme concentration did not significantly affect simulated starch digestion. As such, 15 U/mL_{chyme} was the selected α -amylase concentration for the following experiments (as per Table 1). Interestingly, a lag phase of 10 min, where measured concentration of reducing sugars was below 2 mM, was observed in all cases. The saturation plateau was reached after approximately 30 min.

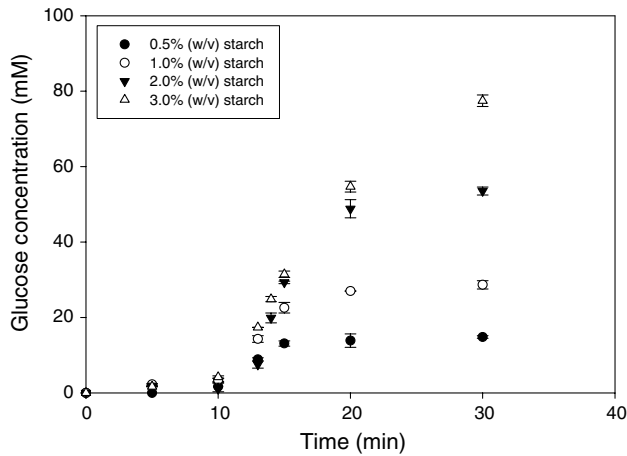


Fig. 7 Starch digestion in the lumen side of the SIM, varying the concentration of starch: (a) 0.5, (b) 1.0, (c) 2.0 and (d) 3.0 % (w/v). Pancreatic solution containing 15 U/mL of α -amylase and 0.5 U/mL of amyloglucosidase was fed at 3.0 mL/min

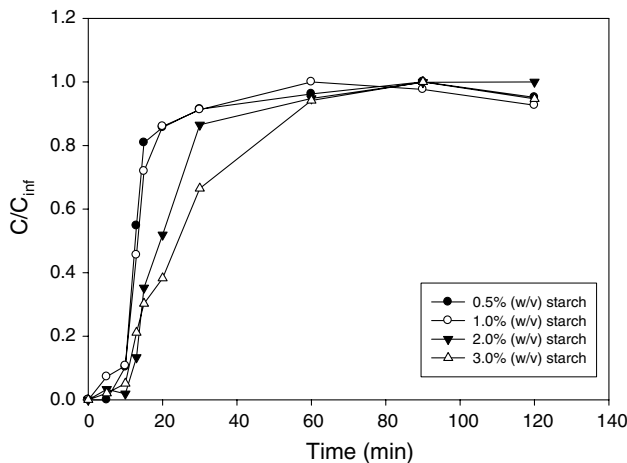


Fig. 8 Starch digestion in the lumen side of the SIM, varying the concentration of starch from 0.5 to 3.0 % (w/v)

Effect of substrate concentration

Figure 7 shows starch hydrolysis in the SIM at various initial starch concentrations (experiment 3 in Table 1). Similarly to previous experiments with the SIM, a 10-min lag phase is observed for all investigated conditions, where practically no reaction can be observed. Following the lag phase, the amount of hydrolysed starch increases at higher starch content, as the available substrate increases.

For direct comparison between the different conditions, Fig. 8 shows the extent of hydrolysis (concentration of reducing sugars divided by the concentration of the plateau concentration in Fig. 7) with time at the different initial starch contents. As Fig. 8 indicates, the relative rate

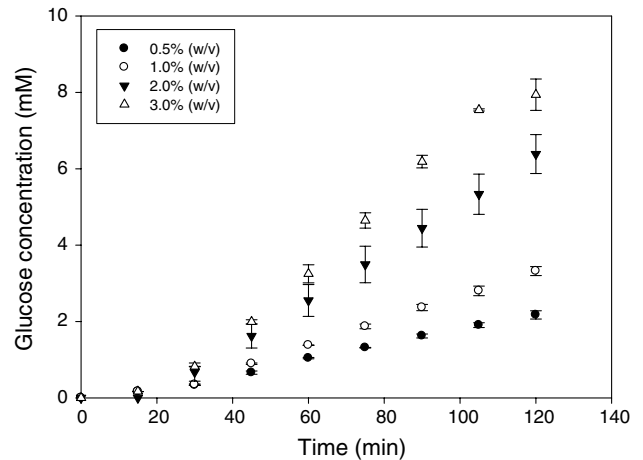


Fig. 9 Glucose absorption in the recipient side of the SIM, varying the concentration of starch from 0.5 to 3.0 % (w/v)

Table 2 Kinetic constant based on first-order reaction behaviour

Model	Mixing method	Kinetic constant, k (min ⁻¹)
STR	Impeller (300 rpm)	0.052
SIM	Segmentation contractions	0.012

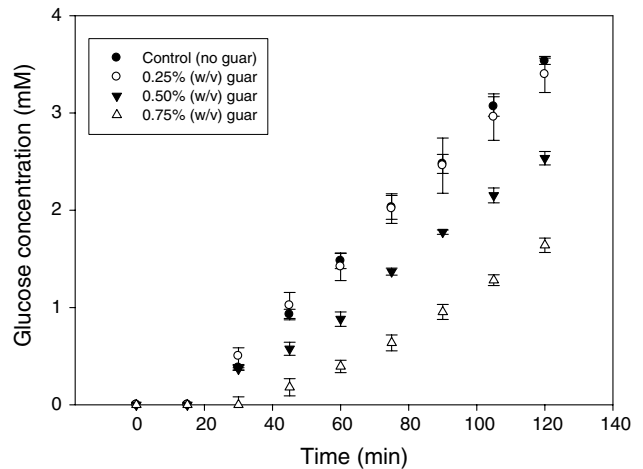


Fig. 10 Glucose absorption in the recipient side of the SIM, varying the concentration of guar gum from 0.25 to 0.75 % (w/v)

of hydrolysis decreases on increasing starch concentration. In addition, the time to reach equilibrium was 30 min for 0.5–2.0 % w/v initial starch content, whereas 60 min were required at 3.0 % starch.

Figure 9 presents results of simulated glucose absorption in the recipient side of the SIM at different initial starch concentrations. As expected, the rise in glucose concentration was greater at increasing starch content due to the higher luminal glucose content. Similarly to observations

Table 3 Rate of absorption at various concentrations of guar gum

Guar concentration (% w/v)	Absorption (10^{-7} M/s)
0.00	5.84 ± 0.12
0.25	5.42 ± 0.52
0.50	3.74 ± 0.17
0.75	3.26 ± 0.34

of “Effect of enzyme concentration” section, absorption of approximately 10 % of the total reducing sugars generated in the luminal side (as defined in Fig. 7) after 2 h of digestion was measured. A 15-min lag phase was observed on all occasions.

Effect of viscosity of the digesta

Samples of 1.0 % starch with addition of guar gum were digested in the SIM (experiment 4 in Table 1). Due to the opacity of the investigated systems, restricting colorimetric analysis of the luminal material, starch hydrolysis was not monitored and simulated glucose absorption was the only parameter measured.

Figure 10 indicates significantly reduced simulated glucose absorption in the recipient side of the SIM for starch solutions (1 %) at increasing guar gum concentrations. Indicatively, on increasing guar gum concentration from 0.5 to 0.75 % (zero shear viscosities 0.3 and 7.0 Pa s, respectively, data not shown) glucose concentration after 2 h of digestion was practically halved (45 % reduction). The associated lag phase increased on increasing viscosity, reaching 30 min for 0.75 % guar gum. Absorption rates obtained from Fig. 10 with linear regression (the lag phases were ignored in these calculations) are tabulated in Table 3. In agreement with the work of Dhital et al. [80], the effect of viscosity on starch digestion was marginal at low viscosities and an evident decrease in simulated glucose absorption on increase in viscosity was only observed at guar gum concentrations above 0.5 %, corresponding to zero shear viscosity of 0.3 Pa s.

Bread experiments

In order to further investigate the effect of food formulation on simulated glucose absorption in the SIM, a real food (white bread) was digested with and without 0.5 % guar gum. Figure 11 shows a 35 % reduction in glucose concentration in the recipient side after 2 h of digestion on inclusion of guar gum. Interestingly, unlike the linear absorption curves obtained for starch systems (Fig. 10), the shape of the bread absorption curve shows a change in slope after approximately 1 h from the beginning of the digestion

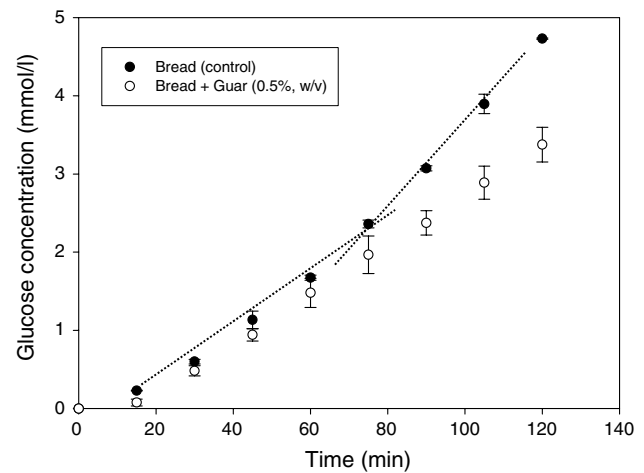


Fig. 11 Simulated glucose absorption curve for 50 g of white bread with and without (control) 0.5 % guar gum

(Fig. 11). This is indicated by a twofold increase in the rate of glucose increase from 0.47 to 0.89 $\mu\text{M/s}$.

Discussion

This work presents results of simulated starch digestion using a novel in vitro small intestinal model (SIM) that considers engineering aspects of digestion (e.g. gut motility, enzymatic flow rate) alongside biochemical (e.g. enzyme concentrations). First the model was compared with the vigorously mixed STR (Fig. 4). A profound difference between the two mixing mechanisms is the presence of a “lag phase” in the SIM, which was absent when the STR was used instead. In the STR, mixing is fully turbulent (indicated by a high Reynolds number, $Re = 11,300$), apparent starch hydrolysis is fast, and saturation is reached within 20 min. By contrast, 40 min (double the time) is required to reach equilibrium in the SIM, where mass transfer is facilitated by the more physiologically representative simulated segmentation contractions, and mixing falls within the transitional regime from laminar to fully turbulent (indicated by a lower Reynolds number of 2100). This “lag phase” may therefore be the result of slow, non-homogeneous mixing in the lumen, which significantly reduced initial apparent rate of hydrolysis due to mass transfer limitations between starch and enzyme molecules. This is the time required for the enzyme to meet with the substrate so that the reaction can occur. The presence of lag phase will have a significant physiological effect on slowing the rate of starch digestion in the gut and the associated rise in postprandial blood glucose levels.

The significance of mixing on simulated starch hydrolysis is further quantified when comparing the reaction kinetic constants (Table 2): 4.5 times higher constants were estimated for the STR, compared to the SIM, reaching 0.05 min^{-1} . It is noted that similar constants (of the order of 0.04 min^{-1}) have been reported for in vitro digestion of starchy foods using biochemical (batch) models with fast, homogeneous mixing conditions [40, 41]. The difference with SIM may indicate a way of controlling glucose bioaccessibility after consumption of starchy foods by controlling the mixing processes in the gut.

The model was further used to evaluate the effect of enzyme flow rate, enzyme concentration, starch (substrate) concentration, and viscosity (addition of guar gum) on starch digestion. Overall, the results highlighted the importance of mass transfer on digestive processes. On all occasions, the presence of lag phase (of the order of 10–15 min) was evident on both starch hydrolysis and simulated glucose absorption. At low enzymatic flow rates, slower digestion was observed until a critical level (3.0 mL/min) was reached, above which any further increase marginally affected digestion kinetics (Fig. 5). It appears, therefore, that at higher flow rates mass transfer ceases to be a limiting factor in the apparent rate of reactions. It is interesting to note that this number falls within the physiological range of $4.13 \pm 0.88 \text{ mL/min}$ reported by [77] (see also “Effect of enzyme flow rate; enzyme concentration; starch concentration; chyme viscosity” section). Similar pattern was observed when the independent variable was enzyme concentration. Increasing amylase content resulted in faster rates of digestion until a critical value (of $15 \text{ U/mg}_{\text{chyme}}$) was reached. Any further increase in amylase activity had an insignificant effect on digestion kinetics under investigated conditions (Fig. 6).

The viscosity of the digested system appeared to have a critical role on digestion kinetics. Indicatively, 50 % reduced simulated glucose absorption was observed on addition of a thickener (0.75 % guar gum) after 2 h of digestion (Fig. 10), and approximately 30 % reduction in the extent of starch digestion after 40 min was measured on increase in starch concentration from 1 to 3 % (Fig. 8). It is noted that systems with higher starch concentration exhibited higher viscosities. A possible mechanism of action for this reduction observed in the simulated starch digestion is through the mass transfer limitations induced as viscosity increases. These trends are consistent with in vivo findings that viscous fibres, guar gum in particular, reduce postprandial glucose levels after consumption of preparations with similar glucose concentrations [17, 81].

Having established the importance of mass transfer, gut motility, and digestive conditions on digestibility of starch, a more complex starchy food was selected for further studies. Simulated glucose absorption after digestion of bread

showed an interesting and unexpected trend: the initial rate of digestion was followed by a faster (almost doubled) rate after minute 80 (Fig. 11). It is possible that this increase is related to a decrease in the luminal viscosity as the bread is being hydrolysed, serving as an additional indicator of the strong link between mass transfer during digestion and associated postprandial response. Any solid conclusions are preliminary at this stage, and further work is required to determine the effect of digestion on the rheological properties of chyme. It is noted, however, that addition of 0.5 % guar gum resulted in reduced mass transfer and reduced (by 35 %) simulated glucose absorption after 2 h of simulated intestinal digestion.

Conclusions

The importance of mass transfer and gut motility in nutritional studies was considered. Typically, in vitro nutritional studies are performed in small volumes and the effect of segmentation in the small intestine is often disregarded. Using a novel in vitro small intestinal model (SIM) with realistic representation of segmentation contractions, digestion of starch and starchy foods (bread) showed increased lag phase before any hydrolysis could be observed, compared to digestion using rigorous, homogeneous mixing. In addition, increased lag phase and reduced mass transfer were obtained on increasing chyme viscosity. Decreased mass transfer appeared to reduce both starch hydrolysis rates and nutrient bioaccessibility. Overall, this work illustrates the potential of controlling food digestion and postprandial response, including blood glucose levels, by engineering food structures with the desired behaviour in the small intestine for the benefit of public health.

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References

1. Bidlack WR (1996) Interrelationships of food, nutrition, diet and health: the National Association of State Universities and Land Grant Colleges White Paper. *J Am Coll Nutr* 15:422–433
2. Bodinham CL, Frost GS, Robertson MD (2010) Acute ingestion of resistant starch reduces food intake in healthy adults. *Br J Nutr* 103:917–922. doi:10.1017/S0007114509992534
3. Fiszman S, Varela P (2013) The role of gums in satiety/satiation. A review. *Food Hydrocoll* 32:147–154. doi:10.1016/j.foodhyd.2012.12.010
4. Gariballa SE (2007) Nutritional factors in stroke. *Br J Nutr* 84:5. doi:10.1017/S0007114500001173

5. Gidley MJ (2013) Hydrocolloids in the digestive tract and related health implications. *Curr Opin Colloid Interface Sci* 18:371–378. doi:10.1016/j.cocis.2013.04.003
6. Kaufmann SFM, Palzer S (2011) Food structure engineering for nutrition, health and wellness. *Procedia Food Sci* 1:1479–1486. doi:10.1016/j.profoo.2011.09.219
7. Lentle RG, Janssen PWM (2008) Physical characteristics of digesta and their influence on flow and mixing in the mammalian intestine: a review. *J Comp Physiol B* 178:673–690. doi:10.1007/s00360-008-0264-x
8. Anandacoomarasamy A, Caterson I, Sambrook P et al (2008) The impact of obesity on the musculoskeletal system. *Int J Obes (Lond)* 32:211–222. doi:10.1038/sj.ijo.0803715
9. Larsen SH, Wagner G, Heitmann BL (2007) Sexual function and obesity. *Int J Obes (Lond)* 31:1189–1198. doi:10.1038/sj.ijo.0803604
10. Profenno LA, Porsteinsson AP, Faraone SV (2010) Meta-analysis of Alzheimer's disease risk with obesity, diabetes, and related disorders. *Biol Psychiatry* 67:505–512. doi:10.1016/j.biopsych.2009.02.013
11. Suk S-H, Sacco RL, Boden-Albala B et al (2003) Abdominal obesity and risk of ischemic stroke: the Northern Manhattan Stroke Study. *Stroke* 34:1586–1592. doi:10.1161/01.STR.0000075294.98582.2F
12. House of Commons - Health - Third Report. <http://www.publications.parliament.uk/pa/cm200304/cmselect/cmhealth/23/2302.htm>. Accessed 19 Jan 2015
13. Tackling obesities: future choices - GOV.UK. <https://www.gov.uk/government/collections/tackling-obesities-future-choices>. Accessed 19 Jan 2015
14. Guyton AC, Hall JE (2006) *Textbook of medical physiology*. Elsevier Saunders, Philadelphia
15. Lundin L, Golding M, Wooster TJ (2008) Understanding food structure and function in developing food for appetite control. *Nutr Diet* 65:S79–S85. doi:10.1111/j.1747-0080.2008.00266.x
16. Juntunen KS, Niskanen LK, Liukkonen KH et al (2002) Postprandial glucose, insulin, and incretin responses to grain products in healthy subjects. *Am J Clin Nutr* 75:254–262
17. Jenkins DJ, Wolever TM, Leeds AR et al (1978) Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. *Br Med J* 1:1392–1394
18. Norton AB, Cox PW, Spyropoulos F (2011) Acid gelation of low acyl gellan gum relevant to self-structuring in the human stomach. *Food Hydrocoll* 25:1105–1111. doi:10.1016/j.foodhyd.2010.10.007
19. Bradbeer JF, Hancocks R, Spyropoulos F, Norton IT (2014) Self-structuring foods based on acid-sensitive low and high acyl mixed gellan systems to impact on satiety. *Food Hydrocoll* 35:522–530. doi:10.1016/j.foodhyd.2013.07.014
20. Wilde PJ, Chu BS (2011) Interfacial and colloidal aspects of lipid digestion. *Adv Colloid Interface Sci* 165:14–22. doi:10.1016/j.cis.2011.02.004
21. Wong S, Traianedes K, O'Dea K (1985) Factors affecting the rate of hydrolysis of starch in legumes. *Am J Clin Nutr* 42:38–43
22. Lehmann U, Robin F (2007) Slowly digestible starch—its structure and health implications: a review. *Trends Food Sci Technol* 18:346–355. doi:10.1016/j.tifs.2007.02.009
23. Englyst KN, Englyst HN (2007) Carbohydrate bioavailability. *Br J Nutr* 94:1. doi:10.1079/BJN20051457
24. Slaughter SL, Ellis PR, Butterworth PJ (2001) An investigation of the action of porcine pancreatic α -amylase on native and gelatinised starches. *Biochim Biophys Acta Gen Subj* 1525:29–36. doi:10.1016/S0304-4165(00)00162-8
25. Ells LJ, Seal CJ, Kettlitz B et al (2007) Postprandial glycaemic, lipaemic and haemostatic responses to ingestion of rapidly and slowly digested starches in healthy young women. *Br J Nutr* 94:948. doi:10.1079/BJN20051554
26. Englyst HN, Kingman SM, Cummings JH (1992) Classification and measurement of nutritionally important starch fractions. *Eur J Clin Nutr* 46(Suppl 2):S33–S50
27. Shu X, Jia L, Ye H et al (2009) Slow digestion properties of rice different in resistant starch. *J Agric Food Chem* 57:7552–7559. doi:10.1021/jf900988h
28. Zhang G, Ao Z, Hamaker BR (2006) Slow digestion property of native cereal starches. *Biomacromolecules* 7:3252–3258. doi:10.1021/bm060342i
29. Ao Z, Simsek S, Zhang G et al (2007) Starch with a slow digestion property produced by altering its chain length, branch density, and crystalline structure. *J Agric Food Chem* 55:4540–4547. doi:10.1021/jf063123x
30. Chung H-J, Shin D-H, Lim S-T (2008) In vitro starch digestibility and estimated glycemic index of chemically modified corn starches. *Food Res Int* 41:579–585. doi:10.1016/j.foodres.2008.04.006
31. Leloup VM, Colonna P, Ring SG (1991) Alpha-amylase adsorption on starch crystallites. *Biotechnol Bioeng* 38:127–134. doi:10.1002/bit.260380204
32. Snow P, O'Dea K (1981) Factors affecting the rate of hydrolysis of starch in food. *Am J Clin Nutr* 34:2721–2727
33. Wolf BW, Bauer LL, Fahey GC (1999) Effects of chemical modification on in vitro rate and extent of food starch digestion: an attempt to discover a slowly digested starch. *J Agric Food Chem* 47:4178–4183. doi:10.1021/jf9813900
34. Zhou Z, Topping DL, Morell MK, Bird AR (2010) Changes in starch physical characteristics following digestion of foods in the human small intestine. *Br J Nutr* 104:573–581. doi:10.1017/S0007114510000875
35. Willis HJ, Eldridge AL, Beiseigel J et al (2009) Greater satiety response with resistant starch and corn bran in human subjects. *Nutr Res* 29:100–105. doi:10.1016/j.nutres.2009.01.004
36. Chandalia M, Garg A, Lutjohann D et al (2000) Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. *N Engl J Med* 342:1392–1398. doi:10.1056/NEJM200005113421903
37. McIntosh M, Miller C (2001) A diet containing food rich in soluble and insoluble fiber improves glycemic control and reduces hyperlipidemia among patients with type 2 diabetes mellitus. *Nutr Rev* 59:52–55
38. Rave K, Roggen K, Dellweg S et al (2007) Improvement of insulin resistance after diet with a whole-grain based dietary product: results of a randomized, controlled cross-over study in obese subjects with elevated fasting blood glucose. *Br J Nutr* 98:929–936. doi:10.1017/S0007114507749267
39. Truswell AS (1992) Glycaemic index of foods. *Eur J Clin Nutr* 46(Suppl 2):S91–S101
40. Frei M, Siddhuraju P, Becker K (2003) Studies on the in vitro starch digestibility and the glycemic index of six different indigenous rice cultivars from the Philippines. *Food Chem* 83:395–402. doi:10.1016/S0308-8146(03)00101-8
41. Goñi I, Garcia-Alonso A, Saura-Calixto F (1997) A starch hydrolysis procedure to estimate glycemic index. *Nutr Res* 17:427–437. doi:10.1016/S0271-5317(97)00010-9
42. Li J, Vasanthan T, Hoover R, Rossnagel B (2004) Starch from hull-less barley: V. In vitro susceptibility of waxy, normal, and high-amylose starches towards hydrolysis by alpha-amylases and amyloglucosidase. *Food Chem* 84:621–632. doi:10.1016/S0308-8146(03)00287-5
43. McClements DJ, Li Y (2010) Review of in vitro digestion models for rapid screening of emulsion-based systems. *Food Funct* 1:32–59. doi:10.1039/c0fo00111b

44. Minekus M, Alminger M, Alvito P et al (2014) A standardised static in vitro digestion method suitable for food—an international consensus. *Food Funct* 5:1113–1124. doi:[10.1039/c3fo60702j](https://doi.org/10.1039/c3fo60702j)
45. Ménard O, Cattenoz T, Guillemin H et al (2014) Validation of a new in vitro dynamic system to simulate infant digestion. *Food Chem* 145:1039–1045. doi:[10.1016/j.foodchem.2013.09.036](https://doi.org/10.1016/j.foodchem.2013.09.036)
46. Gouseti O, Jaime-Fonseca MR, Fryer PJ et al (2014) Hydrocolloids in human digestion: dynamic in vitro assessment of the effect of food formulation on mass transfer. *Food Hydrocoll.* doi:[10.1016/j.foodhyd.2014.06.004](https://doi.org/10.1016/j.foodhyd.2014.06.004)
47. Guerra A, Etienne-Mesmin L, Livrelli V et al (2012) Relevance and challenges in modeling human gastric and small intestinal digestion. *Trends Biotechnol* 30:591–600. doi:[10.1016/j.tibtech.2012.08.001](https://doi.org/10.1016/j.tibtech.2012.08.001)
48. Lea AS (1889) A comparative study of natural and artificial digestions (preliminary account). *Proc R Soc Lond* 47:192–197. doi:[10.2307/114935](https://doi.org/10.2307/114935)
49. Benjamin O, Silcock P, Kieser JA et al (2012) Development of a model mouth containing an artificial tongue to measure the release of volatile compounds. *Innov Food Sci Emerg Technol* 15:96–103. doi:[10.1016/j.ifset.2012.03.004](https://doi.org/10.1016/j.ifset.2012.03.004)
50. De Loubens C, Panouillé M, Saint-Eve A et al (2011) Mechanistic model of in vitro salt release from model dairy gels based on standardized breakdown test simulating mastication. *J Food Eng* 105:161–168. doi:[10.1016/j.jfoodeng.2011.02.020](https://doi.org/10.1016/j.jfoodeng.2011.02.020)
51. Lvova L, Denis S, Barra A et al (2012) Salt release monitoring with specific sensors in “in vitro” oral and digestive environments from soft cheeses. *Talanta* 97:171–180. doi:[10.1016/j.talanta.2012.04.013](https://doi.org/10.1016/j.talanta.2012.04.013)
52. Mills T, Spyropoulos F, Norton IT, Bakalis S (2011) Development of an in vitro mouth model to quantify salt release from gels. *Food Hydrocoll* 25:107–113. doi:[10.1016/j.foodhyd.2010.06.001](https://doi.org/10.1016/j.foodhyd.2010.06.001)
53. Salles C, Tarrega A, Mielle P et al (2007) Development of a chewing simulator for food breakdown and the analysis of in vitro flavor compound release in a mouth environment. *J Food Eng* 82:189–198. doi:[10.1016/j.jfoodeng.2007.02.008](https://doi.org/10.1016/j.jfoodeng.2007.02.008)
54. Chen J, Gaikwad V, Holmes M et al (2011) Development of a simple model device for in vitro gastric digestion investigation. *Food Funct* 2:174–182. doi:[10.1039/c0fo00159g](https://doi.org/10.1039/c0fo00159g)
55. Kong F, Singh RP (2008) A model stomach system to investigate disintegration kinetics of solid foods during gastric digestion. *J Food Sci* 73:E202–E210. doi:[10.1111/j.1750-3841.2008.00745.x](https://doi.org/10.1111/j.1750-3841.2008.00745.x)
56. Lo Curto A, Pitino I, Mandalari G et al (2011) Survival of probiotic lactobacilli in the upper gastrointestinal tract using an in vitro gastric model of digestion. *Food Microbiol* 28:1359–1366. doi:[10.1016/j.fm.2011.06.007](https://doi.org/10.1016/j.fm.2011.06.007)
57. Mercuri A, Lo Curto A, Wickham MSJ et al (2008) Dynamic gastric model (DGM): a novel in vitro apparatus to assess the impact of gastric digestion on the droplet size of self-emulsifying drug-delivery systems. *J. Pharm. Pharmacol*
58. Vardakou M, Mercuri A, Barker SA et al (2011) Achieving antral grinding forces in biorelevant in vitro models: comparing the USP dissolution apparatus II and the dynamic gastric model with human in vivo data. *AAPS PharmSciTech* 12:620–626. doi:[10.1208/s12249-011-9616-z](https://doi.org/10.1208/s12249-011-9616-z)
59. Wickham M, Faulks R (2008–2012) Dynamic gastric model, European Patent EP1907108A1 (issued 2008); US Patent US 8,092,222 (issued 2012); Australian Patent AU2006271423 (issued 2012)
60. Tharakan A, Norton IT, Fryer PJ, Bakalis S (2010) Mass transfer and nutrient absorption in a simulated model of small intestine. *J Food Sci* 75:E339–E346. doi:[10.1111/j.1750-3841.2010.01659.x](https://doi.org/10.1111/j.1750-3841.2010.01659.x)
61. Blanquet S, Marol-Bonnin S, Beyssac E et al (2001) The “biodrug” concept: an innovative approach to therapy. *Trends Biotechnol* 19:393–400. doi:[10.1016/S0167-7799\(01\)01739-5](https://doi.org/10.1016/S0167-7799(01)01739-5)
62. Bornhorst GM, Singh RP (2013) Kinetics of in vitro bread bolus digestion with varying oral and gastric digestion parameters. *Food Biophys* 8:50–59. doi:[10.1007/s11483-013-9283-6](https://doi.org/10.1007/s11483-013-9283-6)
63. Hoebler C, Lecannu G, Belleville C et al (2002) Development of an in vitro system simulating bucco-gastric digestion to assess the physical and chemical changes of food. *Int J Food Sci Nutr* 53:389–402. doi:[10.1080/0963748021000044732](https://doi.org/10.1080/0963748021000044732)
64. Mainville I, Arcand Y, Farnworth ER (2005) A dynamic model that simulates the human upper gastrointestinal tract for the study of probiotics. *Int J Food Microbiol* 99:287–296. doi:[10.1016/j.ijfoodmicro.2004.08.020](https://doi.org/10.1016/j.ijfoodmicro.2004.08.020)
65. Marteau P, Minekus M, Havenaar R, Huis in’t Veld JH (1997) Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine: validation and the effects of bile. *J Dairy Sci* 80:1031–1037. doi:[10.3168/jds.S0022-0302\(97\)76027-2](https://doi.org/10.3168/jds.S0022-0302(97)76027-2)
66. Minekus M, Smeets-Peters M, Bernalier A et al (1999) A computer-controlled system to simulate conditions of the large intestine with peristaltic mixing, water absorption and absorption of fermentation products. *Appl Microbiol Biotechnol* 53:108–114. doi:[10.1007/s002530051622](https://doi.org/10.1007/s002530051622)
67. Duchateau GSMJE, Klaffke W (2008) Product composition, structure, and bioavailability. *Food Biophys* 3:207–212. doi:[10.1007/s11483-008-9076-5](https://doi.org/10.1007/s11483-008-9076-5)
68. Minekus M, Jelier M, Xiao J-Z et al (2005) Effect of partially hydrolyzed guar gum (PHGG) on the bioaccessibility of fat and cholesterol. *Biosci Biotechnol Biochem* 69:932–938. doi:[10.1271/bbb.69.932](https://doi.org/10.1271/bbb.69.932)
69. Blanquet-Diot S, Soufi M, Rambeau M et al (2009) Digestive stability of xanthophylls exceeds that of carotenes as studied in a dynamic in vitro gastrointestinal system. *J Nutr* 139:876–883. doi:[10.3945/jn.108.103655](https://doi.org/10.3945/jn.108.103655)
70. Mateo Anson N, van den Berg R, Havenaar R et al (2009) Bioavailability of ferulic acid is determined by its bioaccessibility. *J Cereal Sci* 49:296–300. doi:[10.1016/j.jcs.2008.12.001](https://doi.org/10.1016/j.jcs.2008.12.001)
71. Chambers SJ, Wickham MSJ, Regoli M et al (2004) Rapid in vivo transport of proteins from digested allergen across sensitized gut. *Biochem Biophys Res Commun* 325:1258–1263. doi:[10.1016/j.bbrc.2004.10.161](https://doi.org/10.1016/j.bbrc.2004.10.161)
72. Mandalari G, Tomaino A, Rich GT et al (2010) Polyphenol and nutrient release from skin of almonds during simulated human digestion. *Food Chem* 122:1083–1088. doi:[10.1016/j.foodchem.2010.03.079](https://doi.org/10.1016/j.foodchem.2010.03.079)
73. Dupont D, Mandalari G, Mollé D et al (2010) Food processing increases casein resistance to simulated infant digestion. *Mol Nutr Food Res* 54:1677–1689. doi:[10.1002/mnfr.200900582](https://doi.org/10.1002/mnfr.200900582)
74. Thomas K, Aalbers M, Bannon GA et al (2004) A multi-laboratory evaluation of a common in vitro pepsin digestion assay protocol used in assessing the safety of novel proteins. *Regul Toxicol Pharmacol* 39:87–98. doi:[10.1016/j.yrtph.2003.11.003](https://doi.org/10.1016/j.yrtph.2003.11.003)
75. Woolnough JW, Monro JA, Brennan CS, Bird AR (2008) Simulating human carbohydrate digestion in vitro : a review of methods and the need for standardisation. *Int J Food Sci Technol* 43:2245–2256. doi:[10.1111/j.1365-2621.2008.01862.x](https://doi.org/10.1111/j.1365-2621.2008.01862.x)
76. Zhang B, Dhital S, Gidley MJ (2013) Synergistic and antagonistic effects of α -Amylase and amyloglucosidase on starch digestion. *Biomacromolecules* 14:1945–1954. doi:[10.1021/bm400332a](https://doi.org/10.1021/bm400332a)
77. Gregg JA, Sharma MM (1978) Endoscopic measurement of pancreatic juice secretory flow rates and pancreatic secretory pressures after secretin administration in human controls and in patients with acute relapsing pancreatitis, chronic pancreatitis, and pancreatic cancer. *Am J Surg* 136:569–574

78. Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* 31:426–428. doi:10.1021/ac60147a030
79. Levenspiel (1999) Chemical reaction engineering. <http://www.scribd.com/doc/90339373/Levenspiel-1999-Chemical-Reaction-Engineering>. Accessed 19 Jan 2015
80. Dhital S, Dolan G, Stokes JR, Gidley MJ (2014) Enzymatic hydrolysis of starch in the presence of cereal soluble fibre polysaccharides. *Food Funct* 5:579–586. doi:10.1039/c3fo60506j
81. Blackburn NA, Redfern JS, Jarjis H et al (1984) The mechanism of action of guar gum in improving glucose tolerance in man. *Clin Sci (Lond)* 66:329–336