Mathematical modelling of food hydrolysis during in vitro digestion: From single nutrient to complex foods in static and dynamic conditions

Steven Le Feunteun, Sarah Verkempinck, Juliane Floury, Anja Janssen, Alain Kondjoyan, Sebastien Marze, Pierre-Sylvain Mirade, Anton Pluschke, Jason Sicard, George van Aken, et al.

Mathematical modelling of food hydrolysis during *in vitro* digestion: from single nutrient to complex foods in static and dynamic conditions

Steven Le Feunteun[1,*], Sarah Verkempinck[2], Juliane Floury[1], Anja Janssen[3], Alain Kondjoyan[4], Sebastien Marze[5], Pierre-Sylvain Mirade[4], Anton Pluschke[6], Jason Sicard[4], George van Aken[7], Tara Grauwet[2]

[1] STLO, INRAE, Institut Agro, 35042 Rennes, France

ORCID: 0000-0002-4476-2811 (Steven Le Feunteun); 0000-0001-8030-3690 (Juliane Floury)

[2] KU Leuven, Department of Microbial and Molecular Systems (M2S), Laboratory of Food Technology and Leuven Food Science and Nutrition Research Centre (LFoRCe), Kasteelpark Arenberg 23, PB 2457, 3001 Leuven, Belgium

ORCID: 0000-0002-5156-4792 (Tara Grauwet); 0000-0003-0202-4383 (Sarah Verkempinck)

[3] Food Process Engineering, Wageningen University, Bornse Weilanden 9, 6708 WG Wageningen, the Netherlands

ORCID: 0000-0003-2231-7597

[4] INRAE, QuaPA, 63122 Saint-Genès-Champanelle, France

ORCID: 0000-0002-7197-8212 (Jason Sicard); 0000-0003-0636-3008 (Alain Kondjoyan)

[5] INRAE, BIA, 44316 Nantes, France

[6] VF Nutrition GmbH, Mollstr. 32, 10249 Berlin, Germany

ORCID: 0000-0002-6922-8535

[7] Royal Cosun, Cosun innovation center, Kreekweg 1, 4670 VA Dinteloord, The Netherlands

* author whom correspondence should be addressed: steven.le-feunteun@inrae.fr

Abstract

Background: *In vitro* digestion methods are widely used to investigate the effect of food properties on the hydrolysis of the main macronutrients: starch, lipid and protein. The growing quantity of experimental data calls for strategies to quantitatively compare the effect of food composition and structure on their hydrolysis kinetics. Mathematical modelling is a powerful tool for this purpose as it allows to summarize complex phenomena into a few equations, and quantify relevant model parameters.

Scope and Approach: This review focuses on modelling *in vitro* digestion data, more particularly the hydrolysis of the main macronutrients at the gastric and small intestinal stages. Both static and dynamic *in vitro* conditions are considered, giving an overview of the modelling strategies available for each macronutrient. Besides, ongoing efforts to model the effects of food micro- and macrostructure as well as the interplay between macronutrient hydrolysis are summarized. A view on how modelling may help to bridge the gap between *in vitro* and *in vivo* studies is also provided.

Key Findings and Conclusions: *In vitro* digestion and mathematical modelling are highly complementary methods. Mathematical models can provide a full and quantitative picture of the phenomena taking place, meanwhile *in vitro* experiments offer an excellent framework to test modelling concepts and assumptions. Some hybrid strategies, combining *in vitro* and *in silico* approaches have also been proposed to more accurately translate *in vitro* observations into *in vivo* predictions. Although very young, this field of research appears very promising to complement, or offer an alternative to experimental studies.

Keywords: Digestion, Enzymatic hydrolysis, Bioaccessibility, In silico, In vitro, Modelling.

Table of contents

1. Introduction

Understanding the fate of foods during digestion has become a very active field in the food science community. The kinetics of macronutrient hydrolysis, of nutrient bioaccessibility and bioavailability have drawn particular attention as it is well established that slow *versus* fast absorption of nutrients can have important metabolic effects, beneficial or deleterious depending on the nutrient and the nutritional status of the host (Dupont et al., 2018). A large number of studies therefore focus on the effect of food composition and/or structure on the hydrolysis of the main food macronutrients (Capuano & Janssen, 2021; Marze, 2013): specifically starch, lipid, and protein.

Because of the constraints associated with human and animal experiments, most of the current studies uses *in vitro* models to simulate digestion in the GI tract. These models are well-controlled, in particular static digestion protocols (Brodkorb et al., 2019; Minekus et al., 2014), which offer a very good framework to properly compare the kinetics of macronutrient hydrolysis as, for example, affected by food design factors (e.g. structure, formulation, processing). Moreover, in order to get closer to the physiological reality of digestion, there is an increasing use of (semi-)dynamic *in vitro* protocols. The number of laboratories equipped with sophisticated dynamic devices is growing rapidly, and the INFOGEST network has just published a harmonized semi-dynamic digestion protocol that relies on classical laboratory equipment (Mulet-Cabero et al., 2020). In these more complex experimental set-ups, a number of factors evolve concomitantly in each compartment: the biochemical conditions (*e.g.* pH, enzyme concentrations), the amount of transiting food material, the homogeneity of the content, *etc*. These time-dependent variables generally make the quantitative interpretation of the results much more challenging. In this context, mathematical modelling appears as a unique tool to help quantifying and interpreting the corresponding data, evaluate rate constants, check mass balance, test hypotheses on the digestion mechanisms, *etc*. Yet, mathematical modelling is only scarcely considered for *in vitro* digestion studies, most probably by lack of know-how and/or of knowledge on its added value.

As schematically illustrated in Figure 1, the key objective of this paper is to review efforts taken to combine mathematical modelling with *in vitro* digestion experiments, focussing on the

hydrolysis kinetics of the main macronutrients (starch, lipid and protein) and their main hydrolysis products at the gastric and small intestinal stages (Table 1). It is noteworthy that this review is mostly addressed to researchers from the food digestion research field, and that our intention is not to enter the details of mathematical models. Readers interested in having more details on the equations, assumptions, advantages, and limits of a particular modelling approach are therefore invited to read the quoted citation(s). This review starts with a brief introduction on the most widespread approaches to model the enzymatic hydrolysis of macronutrients. We then consider the case of *in vitro* GI digestion in static conditions, describing the most commonly used mathematical approaches for each macronutrient, and illustrating how mathematical models can be used to test our understanding of the digestion of some complex foods. The benefits and future challenges of combining mathematical modelling with (semi-)dynamic *in vitro* digestion experiments is discussed afterwards, to show that this strategy can be very valuable to address practical considerations such as "Can we model the transit and hydrolysis of macronutrients in (semi-)dynamic conditions?" and "How can mathematical models be useful to deal with heterogeneous digestive contents?". This paper ends with a discussion on these mathematical models built on *in vitro* data and on their usefulness to improve our *in silico* prediction capabilities on the fate of food *in vivo* (Le Feunteun et al., 2020, 2021). To avoid confusion in terms of definitions, Box 1 explains the terms and concepts we used throughout this review.

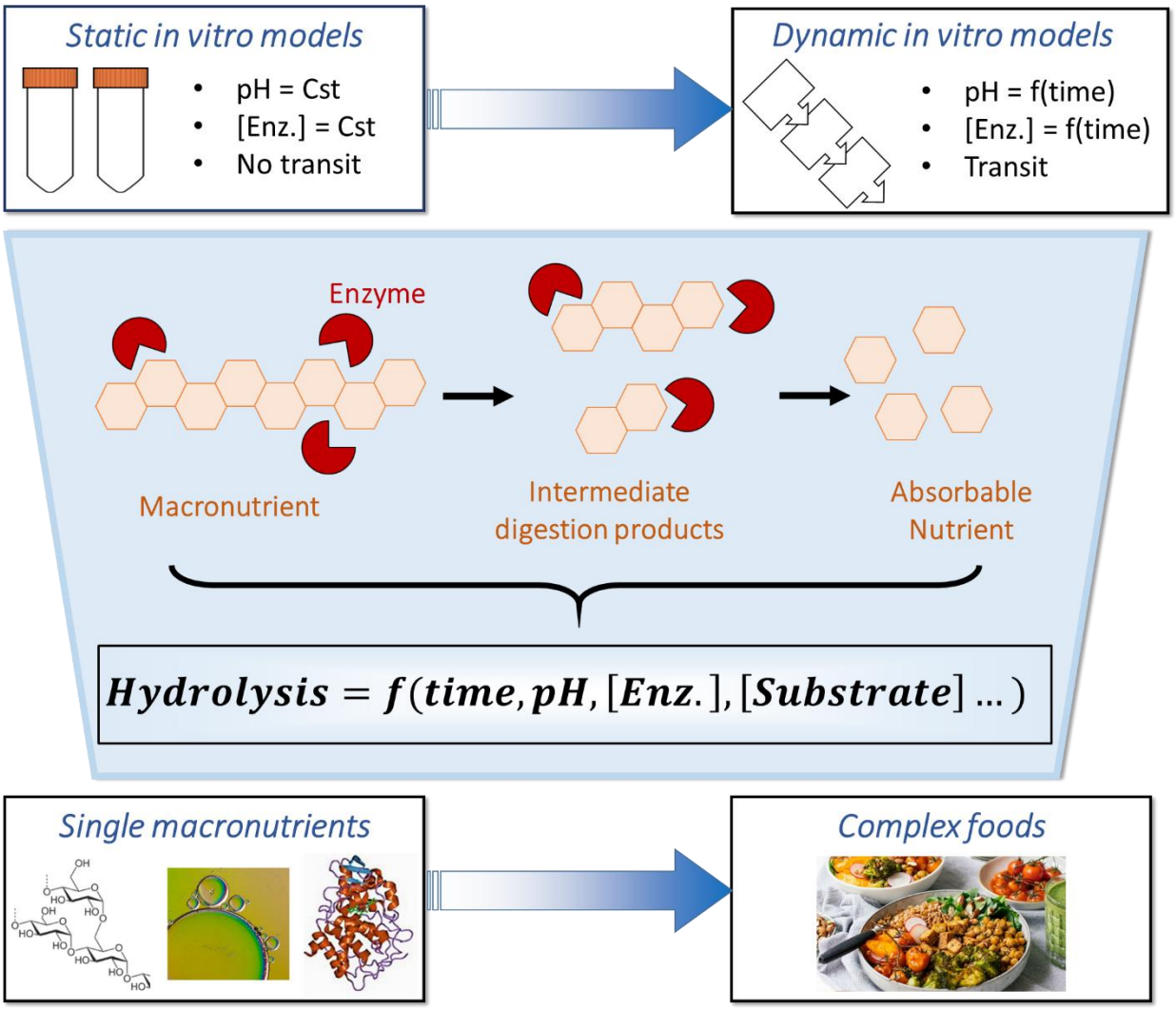

***Figure 1**: Schematic representation of the concepts addressed in this review paper.*

***Table 1**: Main products of macronutrient hydrolysis by gastrointestinal secreted enzymes during the different phases of in vitro digestion. Macronutrients refer to the biopolymers initially present in foods, whereas nutrients refer to the final digestion products typically observed during the different stages of an in vitro digestion (bold). Note that brush border enzymes of the small intestine, which can further hydrolyse tri- and di-peptides, maltose, etc., are not considered in this review.*

Macronutrients	**Main hydrolysis products during *in vitro* digestion**		
	Mouth	*Stomach*	*Small intestine*
Starch	Dextrins	Dextrins, **maltose, maltotriose, α-limit dextrins**	**Maltose, maltotriose, α-limit dextrins**
Protein		Poly- and oligo-peptides	**Tripeptides, dipeptides, amino acids**
Lipid		Diglycerides, **fatty acids**	**Monoglycerides, fatty acids**

Box 1: Definition of frequently used terms and concepts within this review paper.

Nutrient: The term nutrient generally includes both macronutrients and micronutrients. Macronutrients are consumed in large amounts and primary used to deliver energy and be incorporated into tissues, while micronutrients (e.g. vitamins, polyphenols, minerals and other bioactive species) are consumed in smaller amounts but are essential for the organism. For the sake of the present paper however, "nutrient" is defined as the final hydrolysis products of the main macronutrients (starch, lipid, and protein) by gastrointestinal (GI) secreted enzymes (Table 1), as typically studied in *in vitro* digestion studies.

Macronutrient: In the present paper, the term macronutrient refers to the biopolymers initially present in foods (starch, lipid, protein) that need to be hydrolysed by GI secreted enzymes to be converted into nutrients (see above definition). Sugars and fibres, which are not hydrolysed by GI secreted enzymes, are therefore not included in this definition.

Bioaccessibility: Proportion of a nutrient that is chemically and physically available for absorption by the small intestine.

Bioavailability: Proportion of a nutrient that is actually absorbed and is available for functionalisation inside the body.

Static *in vitro* digestion: *In vitro* oral, gastric and/or small intestinal digestion experiments performed under constant physicochemical conditions (constant pH, enzyme concentration, no transit) during each simulated phase.

Semi-dynamic *in vitro* digestion: *In vitro* oral, gastric and/or small intestinal digestion experiments performed with one or several evolving physicochemical condition(s) (evolving pH, and/or enzyme concentration, and/or transit) during one (most generally the gastric phase), but not necessarily all simulated phases.

Dynamic *in vitro* digestion: *In vitro* oral, gastric and/or small intestinal digestion experiments performed with consideration of all the main flux (secretions, transit, and sometimes absorption). These experiments are performed with computer-controlled dynamic *in vitro* digestion systems.

Classic kinetic modelling: Models that are based on the Michaelis-Menten equation, or on assumptions of zero- and first-order kinetic reactions.

Multiresponse modelling: Models that are based on the description of a chemical reaction network, which integrates physical and chemical principles (e.g. chemical equilibrium), in compliance with the definition proposed by van Boekel (2008).

Stochastic modelling: Iterative approach in which each reaction step is described as a discrete event and that relies on the use of one or several random variables (*e.g.* to select a hydrolysable chemical bond, decide whether the selected chemical bond will be cleave by the enzyme or not).

2. Main modelling approaches

As illustrated in Table 2, various approaches may be followed to model the enzymatic hydrolysis of macronutrients such as starch, lipid, and protein. A distinction can be made between the following commonly used modelling approaches: (1) classic kinetic models based on the decrease in substrate concentration and/or the increase in product concentration; (2) multiresponse models based on the description of a common reaction network or a cascade of reactions; (3) stochastic models based on the interactions between substrate and active site of the enzyme using cleavage probabilities.

Classic kinetic models can be used to describe the decrease in substrate concentration or increase in product concentration. For starch, lipid, and protein, product concentration is often related to the degree of hydrolysis (DH), which represents the percentage of broken bonds over the total number of hydrolysable bonds. The substrate/product concentration as a function of time may be described by Michaelis-Menten kinetics (Cornish-Bowden, 2015), and can in most cases be simplified to first-order kinetics to predict the progress of the enzymatic reaction with respect to time. These models are mathematically simple, easy to apply, and are often sufficient to quantitatively describe macronutrient hydrolysis under static *in vitro* digestion conditions.

The breakdown of macronutrients into nutrients is generally not a simple substrate to product conversion. In most cases, a cascade of reactions or a sequence of parallel reactions takes place. Based on mechanistic insights, such a reaction network can be described by a set of differential equations, which integrates physical and chemical principles (e.g. chemical equilibrium) in order to predict experimental concentrations of all reactants and products of this network. This modelling technique is called 'multiresponse kinetic modelling' (van Boekel, 2008). Although these equations look complex, many software programs are available to numerically (i.e. iterative approach) solve them.

Another approach is the use of stochastic models for quantitative predictions of all reactants and products during the course of the hydrolysis reaction. Stochastic modelling, which relies on the use of at least one random variable, has been used for the hydrolysis of starch (Besselink et al., 2008) and protein (Tonda et al., 2017), in which each reaction step is described as a discrete event. The substrate is chosen randomly and binds with the enzyme. Whether the binding is productive or non-productive depends on the enzyme characteristics. Only a productive binding leads to hydrolysis. The enzyme characteristics are implemented based on information on cleavage probability tables (in context of proteases) or subsite maps (in context of amylases). The substrate characteristics are described by the amino acid sequence in case of protein, while for starch, the structure can be described by a two-dimensional array where the glucose units are numbered, depending on the location in the starch molecule.

Overall, various modelling approaches, with different levels of mathematical complexity, can be used to simulate macronutrient hydrolysis. This illustrates that even under well-controlled *in vitro* digestion conditions, kinetic modelling may become much more complex than the classical Michaelis-Menten model for a one-step enzymatic hydrolysis. In the following, we will provide examples of what can be learned from such modelling approaches on the digestion behaviour of starch, lipid, and protein, starting with the case of static *in vitro* digestion conditions.

***Table 2**: Main approaches to model enzymatic hydrolysis kinetics based on static in vitro digestion data.*

Modelling approach	Main assumptions and general form of the equation(s)	Pros	Cons	Enzymatic reaction(s)	Reference
Based on first order reaction model	The reaction rate is assumed proportional to the substrate "accessibility", leading to: $$\frac{dP}{dt} = k\,S$$ where P is the product concentration and k is the rate constant. For starch and protein, S is the substrate concentration. For lipid, S is the surface area of the water-lipid interface. For starch and proteins, this equation has an analytical solution of the form: $$P = P_\infty\,(1 - e^{-kt})$$ with P_∞ the final product concentration. For lipids, an analytical solution can only be obtained under further assumptions on the time evolution of the lipid surface area.	Analytical equations can be derived for simple cases, and these can often be linearized Simple to use, and convenient to model series of reactions and/or parallel reactions Many variants have been proposed (pseudo-first order reaction) enabling different shapes of profile to be reproduced	Model parameters can be difficult to interpret (*e.g.* k depends on enzyme concentration, substrate accessibility, catalytic rate, etc.). Adaptations (pseudo-first order model) are often needed to accurately model experimental data	Small intestinal amylolysis	(Goñi et al., 1997; Butterworth et al., 2012; Edwards et al., 2014; Gwala et al., 2019, 2020; Pallares Pallares et al., 2018, 2019)
				Small intestinal lipolysis	(Lykidis et al., 1995; Y. Li & McClements, 2010; Mitchell et al., 2008; Marze & Choimet, 2012; Giang et al., 2015, 2016; Salvia-Trujillo et al., 2017; Verkempinck, Salvia-Trujillo, Moens, Carrillo, et al., 2018; Verkempinck, Salvia-Trujillo, Moens, Charleer, et al., 2018)
				Gastric & small intestinal proteolysis	(Kondjoyan et al., 2015; Margot et al., 1997; Vorob'ev, 2019)
Based on the Michaelis-Menten equation	The Michalis-Menten relation is built under the steady-state and the reactant stationary assumptions. This relation can be written as: $$\frac{dP}{dt} = \frac{k_{cat}\,E_0\,S}{K_M + S} = \frac{V_{max}\,S}{K_M + S}$$ where V_{max}, which is the product of the catalytic rate constant (k_{cat}) and the enzyme concentration (E_0), is the maximum velocity of the reaction, S is the substrate concentration, and	Analytical equations can be derived under some semi-theoretical or empirical hypotheses, and these can often be linearized	Models parameters are generally unknown and difficult to measure experimentally Assumptions are not always satisfied,	Small intestinal amylolysis	(Mahasukhonthachat et al., 2010)
				Small intestinal lipolysis	(Verger et al., 1973)

	K_M a constant that is specific of the considered enzyme-substrate. It is noteworthy that when $K_M \gg S$, it can be simplified into a first order equation, using: $k = \frac{k_{cat}\, E_0}{K_M}$. Many models of starch and protein hydrolysis kinetics are based on this relation and its variants. For lipid, a comparable relation (though more complex) can be derive to take into account the interfacial nature (*i.e.* 2D) of the lipolysis reaction under further assumptions on the enzyme and substrate concentrations the interface.	Model parameters have mechanistic meanings Simple to use and convenient to model series of reactions and/or parallel reactions Many variants have been proposed enabling different shapes of profile to be reproduced	in particular for complex foods and at long reaction times Adaptations are often needed to accurately model experimental data	Gastric proteolysis	(Luo, Chen, et al., 2018; Ruan et al., 2010)
Multiresponse kinetic models	Multiresponse models are based on one or several system(s) of equations, generally using rate order reaction models. As an example, for the lipolysis in the small intestine, one can consider at least two reactions leading to the same products (FFA): $1\,TAG + 2\,H_2O \overset{k_1}{\rightarrow} 1\,MAG + 2\,FFA$ $1\,TAG + 3\,H_2O \overset{k_2}{\rightarrow} 3\,FFA + 1\,GLY$ In this example, one can write for FFA that: $\frac{dFFA}{dt} = (2\,k_1 + 3\,k_2)\,TAG$ Similar equations can be obtained for all products.	Can be used to model complex reaction schemes, taking into consideration the interrelations between reactions	More complex to build and solve (systems of equations) Require some modelling and programming skills	Gastric lipolysis	(Infantes-Garcia et al., 2020)
				Small intestinal lipolysis	(Verkempinck et al., 2019)
Stochastic models	Stochastic models of enzymatic hydrolysis rely on the use of at least one random variable (e.g. selection of the chemical bond that will be cleaved), and consider each reaction step as a discrete event (iterative process).	Enable to simulate all intermediate reaction products and steps	Complex to build, computationally heavy Require specific programming skills The time is generally not an explicit variable	Starch hydrolysis (microbial α-amylase)	(Besselink et al., 2008)
				Small intestinal lipolysis	(Marze, 2014, 2015)
				Gastric proteolysis	(Tonda et al., 2017)

3. Modelling nutrient hydrolysis and release in static *in vitro* conditions

This section focuses on mathematical models that have been applied to experimental data obtained under static *in vitro* conditions (*i.e.* constant pH and enzyme concentration). It must be noted that the hydrolysis by brush border enzymes is not considered in this discussion because these are only scarcely used *in vitro*, and even less considered in related mathematical models. We first consider the case of food systems that exclusively, or predominantly, consist of one type of macronutrient (*e.g.* starch-rich foods, oil-in-water emulsions, protein solutions). Thereafter, we discuss the case of more complex structured foods, for which the time evolution of the micro- and macro-structure has to be considered.

3.1. The case of single macronutrients

3.1.1. Starch

Two main enzymes contribute to the hydrolysis of starch in the lumen of the human GI tract: salivary and pancreatic α-amylases. Despite the short duration of the oral phase, the *in vivo* literature shows that human salivary α-amylase can hydrolyze up to 9 and 13% of the starch content of bread and spaghetti, respectively, into oligosaccharides (Hoebler et al., 1998). In static *in vitro* digestion protocols, however, the oral phase is often ignored or only performed to break down solid foods into small-sized particles with no addition of salivary α-amylase. This is justified by the short duration of the oral phase and by the widespread use of a gastric pH of 3.0 or below, which irreversibly inactivates human salivary α-amylase (Bernfeld et al., 1948; Freitas & Le Feunteun, 2019). Although some studies have suggested that an oral digestion step should be included in static *in vitro* digestion protocols as it modifies starch digestion kinetics in the intestinal phase (Tamura et al., 2017), others did not observe any modification (Woolnough et al., 2010). For these reasons, it is understandable that no real efforts have been made so far to model starch hydrolysis by salivary α-amylase under static *in vitro* conditions. This may change in the near future, partly due to the increasing use of (semi-)dynamic digestion models (Freitas et al., 2018; Mulet-Cabero et al., 2020).

Most of the experimental work under static conditions focused on starch digestion kinetics during the small intestinal phase (sometimes referred to as 'digestograms'). In the 1990's, it has been shown that such approach can be very powerful to predict the glycemic impact of foods in humans (Englyst et al., 1992; Goñi et al., 1997). As reviewed by others (Dona et al., 2010), several static *in vitro* protocols have been proposed for that purpose, generally leading to comparable successes (Bohn et al., 2018). Nonetheless, caution is advised when analyzing, comparing, and/or modelling starch *in vitro* digestion kinetics from literature due to the large variability of *in vitro* small intestinal conditions used. For instance, some protocols incorporate amyloglucosidase to mimic mucosal α-glucosidases that do not perfectly correspond to those observed using mammalian α-glucosidases (Shin et al., 2019).

Small intestinal starch digestion profiles generally show an exponential behavior, but a rapid-to-slow digestion rate phenomenon is also frequently observed (Butterworth et al., 2012). This is one reason why Englyst, Kingman, and Cummings (1992) have proposed to distinguish the rapidly digestible starch (RDS) fraction from the slowly digestible starch (SDS) and the resistant starch (RS) fractions. To extract the rate(s) and extent of hydrolysis from such digestion profiles, first-order reaction models are most commonly used, though other models have been proposed in the literature, including Michaelis-Menten approaches. Most of these models have been reviewed recently in Nguyen and Sopade (2018), in which the authors also examined the predictabilities of more than ten different approaches by means of comparison with published data. From a curve-fitting point of view, several models enable to fairly reproduce monotonous kinetics as well as rapid-to-slow kinetics. The authors also noted the usefulness of the log of slope (LOS) method (Edwards et al., 2014) in revealing this latter phenomenon.

The amylolysis of native starch is known to be influenced by a number of inherent factors including, but not restricted to, the botanical origin of starch, the amylose-amylopectin ratio, amorphous and crystalline patterns, granule surface, pore characteristics, *etc*. (Tester et al., 2004). As will be discussed in section 3.2, attributes of starch in foods, such as the extent of starch gelatinization and its level of accessibility to digestive enzymes are other key determinants of starch digestion kinetics. Clearly, more efforts should be paid to try incorporating such

measurable properties into current mathematical models. As proposed and discussed by several groups (H. Li et al., 2019; Nguyen & Sopade, 2018), a possible way to tackle this is to consider in the same model the possibility of both sequential and parallel hydrolysis kinetics arising from different starch fractions. Starch hydrolysis is indeed a multi-step process, with a diversity of initial substrate characteristics, and with interrelated generation of intermediate and end products. Newly developed HPLC methods quantifying multiple starch digestion products at the molecular level (e.g. Gwala et al. (2019)) may allow a better understanding of the starch digestion process, and serve as a support for the improvement of current stochastic models (Besselink et al., 2008), and/or the development of a multiresponse model to simultaneously consider multiple interlinked starch digestion species.

3.1.2. Lipid

The hydrolysis of lipid (mainly composed of triglycerides) begins in the stomach with the production of diglycerides and free fatty acids by human gastric lipase, reaching degrees of hydrolysis of about 10 to 25% (Carriere et al., 1993), with a progressive inhibition by the accumulation of free fatty acids at the oil-water interface. Only a few attempts to model gastric lipid digestion were considered based on static *in vitro* digestion experiments because of the scarce use of gastric lipase *in vitro*, the low activity of human gastric lipase at the typical gastric pH of 3, as well as the low availability of efficient and affordable human gastric lipase analogues. Recently, rabbit gastric extract has been suggested to be a good alternative for human gastric lipase (Sams et al., 2016) and has therefore been recommended in the last version of the INFOGEST static *in vitro* protocol (Brodkorb et al., 2019). Since then, Infantes-Garcia et al. (2020) have shown that empirical models, such as a fractional conversion model, may be used to model the decrease in triglyceride concentration as function of gastric digestion time. Moreover, the same authors also presented a multiresponse model capable to fit these gastric lipid digestion data.

Most of the *in vitro* and *in silico* works on lipid hydrolysis have concentrated on the small intestinal phase, where bile salts enable the solubilization of final lipolysis products, monoglycerides and free fatty acids. The most recognized Michaelis-Menten model for lipid hydrolysis, which accounts

for the interfacial nature of the reaction under the assumption that the reaction rate is proportional to the surface area available for lipase adsorption, dates from the 1970's (Verger et al., 1973). Models based on first-order kinetic reactions have also been proposed for triglyceride hydrolysis (Lykidis et al., 1995), later also considering substrate saturation and competition at the interface (Mitchell et al., 2008).

In recent years, new developments based on a physical-chemistry approach have been proposed for the hydrolysis of oil-in-water emulsions. A pioneer pseudo first order kinetic model assuming a constant number of oil droplets of identical size was proposed by Li and McClements (2010), taking into account several physicochemical parameters as well as the time-evolution of the surface area available for lipase adsorption. This model, later corrected by Gaucel, Trelea, and Le Feunteun (2015), has inspired other modelling works, notably to further simulate the solubilization of lipolysis products into the bile salt micellar phase by either considering the total amount (Marze and Choimet 2012) or the individual mass of each fatty acid (Giang et al., 2016). Other modelling frameworks have also been proposed, as for instance using agent-based modelling to simulate the solubilization of a lipophilic vitamin into the bile salt micellar phase during the intestinal digestion of a lipid droplet (Marze, 2014, 2015), and to predict expected behaviors in static (saturation of bile salt micelles) and dynamic (recycling of bile salt micelles) *in vitro* conditions.

Similarly to the case of starch, most of the current modelling developments relies on classic modelling strategies (typically zero- or first order kinetics), with a growing consideration of the sequential and/or parallel events required to gain a more detailed understanding of the key phenomena, and a more general view of their interactions. For example, single response modelling strategies have been applied to multiple lipid hydrolysis species (i.e. triglycerides, monoglycerides, free fatty acids) as a function of small intestinal digestion time (Salvia-Trujillo et al., 2017; Verkempinck, Salvia-Trujillo, Moens, Carrillo, et al., 2018; Verkempinck, Salvia-Trujillo, Moens, Charleer, et al., 2018). These studies illustrate that single response modelling is useful to retrieve quantitative information regarding kinetic parameters of the lipolysis process (i.e. rate and final extent), and facilitates quantitative comparisons of the digestion behaviour of different

simplified food systems. Recently, Verkempinck et al. (2019) showed that a more robust mechanistic multiresponse model could be established by considering that 1 triglyceride may release either 1 monoglyceride and 2 free fatty acids, or 3 free fatty acids and 1 glycerol (i.e. complete hydrolysis). This model, which was validated for 28 independent data sets, could be expanded when more lipid digestion products (i.e. diglycerides) and/or regioisomers are quantified, and possibly fused with previously described approaches accounting for physicochemical parameters related to emulsion characteristics (e.g. lipid composition, droplet size, interfacial composition and properties).

3.1.3. Protein

Protein hydrolysis starts in the stomach, where pepsin cleaves proteins into polypeptides, which are further hydrolyzed into small peptides (e.g. tripeptides, dipeptides) and free amino acids in the small intestine by pancreatic proteases, including trypsin and chymotrypsin. Protein digestion is therefore a complex poly-enzymatic and poly-substrate process with many intermediate peptide species. This makes any mechanistically based kinetic modelling, including multiresponse modelling, difficult to build and apply (Margot et al., 1997). Even stochastic modelling, which may appear better suited to tackle the dynamics of complex biochemical networks (Wilkinson, 2009), faces important challenges if only because the substrate specificity of pepsin reported in the literature (cleavage probability tables) does not fully explain experimentally observed peptides (Tonda et al., 2017).

In practice, more classical approaches of kinetic modelling are, therefore, most generally used. A large number of studies is based on the Michaelis-Menten model to describe the kinetics of soluble protein hydrolysis by pepsin (Ruan, Chi, and Zhang 2010; Luo, Chen, et al. 2018) and pancreatic proteases (Maximova & Trylska, 2015). This approach allows predicting the reaction kinetics by taking into account the effects of the enzyme-to-substrate ratio, the affinity of the protease for the substrate, and several other parameters. However, extensive experimental data are often required to build and validate such models, and a common drawback of Michaelis-Menten models is that they are quite specific for the considered enzyme and substrate. This limits

their use in the area of protein digestion as there is a great diversity of edible proteins and food protein structures.

Another common modelling approach relies on the use of first order reaction models. These often enable good fitting and estimation of the rate and final extent of protein hydrolysis but, as for the hydrolysis of starch and lipid, researchers often resort to this modelling strategy to consider different reaction stages conveniently. For instance, Margot, Flaschel, and Renken (1997) developed a two-parameter kinetic model based on an exponential decrease of trypsin activity with increasing fraction of soluble protein, in order to simulate the tryptic digestion of whey proteins in batch reactors under conditions of moderate enzyme inactivation. Several studies have also shown that the unfolding of globular proteins in the acidic environment of the stomach can be a prerequisite for pepsin hydrolysis, a phenomenon that can be modelled by a two-stage reaction scheme (Herman et al., 2006). For instance, Herman, Gao, and Storer (2006) developed a theoretical pattern with two consecutive exponential steps, one for the protein unfolding and one for protein hydrolysis by pepsin, in order to explore protein unfolding as a limiting factor in the gastric digestion of proteins. Another example can be found in the study of Kondjoyan, Daudin, and Santé-Lhoutellier (2015) who developed a model based on first-order reaction kinetics that accounts for heat-induced change of the number of cleavage sites of myofibrillar proteins, to predict their kinetics of *in vitro* digestion by pepsin. Their model showed good prediction capabilities on how the combined effect of heating time, temperature, enzyme concentration, and pH can affect the extent of hydrolysis.

It is noteworthy that the modelling of *in vitro* protein digestion kinetics is also complicated by the fact that foods are made of mixtures of different proteins with different intrinsic properties, and by the diversity of experimental data that can be used to monitor the progress of the protein hydrolysis reaction. The disappearance of intact proteins can be monitored (e.g. by electrophoresis) but implies a parallel modelling of each of the considered proteins. To avoid such difficulties, models are thus most generally compared to the time-evolution of α-amine groups and/or the degree of hydrolysis (Rutherfurd, 2010). However, because such data is averaged over

all protein molecules, these are not the most appropriate to identify the mechanisms determining the curve shape and final extent of the protein hydrolysis reactions (Deng et al., 2018).

3.2. The case of complex structured foods

In the preceding sections, we have highlighted the key influence of single macronutrient characteristics on their hydrolysis kinetics. As exemplified for starch (Dhital et al., 2017), the rate-limiting step for the case of food systems that are predominantly made of one type of macronutrient is likely to be either the catalytic rate of the reaction or the access of enzyme to its substrate. The catalytic turnover will generally be the rate-limiting for well-solubilized substrates and for substrates that are particularly resistant to enzyme hydrolysis, as typically encountered with unswollen starch and some globular proteins. The substrate accessibility will generally be the rate-limiting step whenever the substrate is included within a particular structure, as it is typically the case for lipids (even in a fine oil-in-water emulsions), protein aggregates, or occluded starch. Therefore, even simple food systems cannot always be viewed as a soup of single macronutrients in a diversity of states. Indeed, real foods have different structures, and are generally granular, semi-solid, or solid materials made of several macronutrients that are intrinsically associated into complex architectures ranging from the molecular, the microscopic, and the macroscopic scales (Capuano & Janssen, 2021; Marze, 2013). In these more complex but more realistic cases, substrate accessibility is clearly the rate-limiting step of the hydrolysis of macronutrients, hence calling for more elaborated modelling developments to explicitly represent the surface area of particles/droplets, the diffusion of digestive fluids into complex structures, etc. Still remaining in the context of static in *vitro* digestion, we will now highlight some key effects of food structure on macronutrient hydrolysis and some corresponding modelling approaches.

3.2.1. Phase separation of liquid foods

A first case that causes important modelling challenges concerns liquid food that is unstable under *in vitro* digestion conditions. A well-known example is the case of milk, which curdles in the acidic condition of the gastric phase. This is due to the fast isoelectric protein aggregation of casein

micelles, possibly coupled with a peptic hydrolysis of the κ-casein layer, resulting in structures ranging from firm gel-like to lose granular structures depending on the milk protein composition and process history (Mulet-Cabero et al., 2019). Another well-known example is the case of oil-in-water emulsions, which may flocculate, coalesce, and even cream during both the gastric (Day et al., 2014; Golding et al., 2011) and small intestinal phases (Giang et al., 2015; Mun et al., 2007), depending on emulsion characteristics. Although it has been shown that the rate of lipolysis can still be modelled from the experimentally determined time-evolution of the surface area of oil droplets during coalescence (Giang et al., 2015), the hypothesis of a constant number of oil droplets of identical size (Li and McClements 2010) does not hold anymore. Protein aggregation/precipitation and oil creaming therefore represent cases where, beyond enzymatic considerations, one would need to predict the phase separation of the substrate within the mathematical models to accurately simulate macronutrient hydrolysis.

3.2.2. Solid foods

For practical purposes, static *in vitro* digestion protocols often recommend to mince solid foods to produce a paste-like consistency after mixing with saliva, with addition of water if necessary (Brodkorb et al., 2019). However, many researchers are interested in performing a more physiologically relevant oral phase, which will typically produce particles of several millimeters in size. This brings up new challenges as the hydrolysis kinetics of the macronutrients contained in the particles will be largely controlled by their size distribution and inner properties.

For compact inner structures, such as those in pasta, digestive enzymes only erode superficially the particles to reach underneath layers. Their rate of digestion therefore mostly depend on their surface area, and hence on the particle size distribution and geometry (Monro et al., 2011). Although performed in unstirred conditions, it is noteworthy that erosion has been suggested to be the predominant mechanism of the enzymatic breakdown of dairy gel particles containing about 65% of moisture (Floury et al., 2018). Providing that *in vivo* gastric motility only induces a gentle mixing of foods, it is thus likely that an erosion mechanism should be considered for many solid foods or gels.

Beyond particle surface area, the capability of the digestive fluids to penetrate the particles is another key parameter to consider. Indeed, for porous structures, such as hydrogels, one also needs to consider the diffusion of the digestive fluids within the particles. This becomes important whenever the time scale of particle digestion is longer than the migration of protons (gastric phase), bicarbonates (intestinal phase), and enzymes. To go beyond an empirical modelling of the macroscopic observations, several approaches have been proposed. For instance, the diffusion of acidic water in carrot or cheese has been modelled using the differential form of Darcy's law, as well as the subsequent soluble solid loss using a differential equation for mass conservation. Taken together, these two laws could model the observed solid loss for both constant and decreasing gastric pH. Diffusion of acid has also been studied experimentally and modelled using Fick's law for rice, red beets, and protein gels during gastric digestion (Luo, Zhan, et al., 2018; Mennah-Govela et al., 2019, 2020; Mennah-Govela et al., 2015), and effective diffusivity values for foods with different macrostructure and buffering capacity have been determined. Those results are of particular interest because the diffusion coefficients of protons, bicarbonates, and enzymes can be found in (or estimated from) the literature for various foods, as for example the diffusion of pepsin in dairy protein gels (Luo et al., 2017; Thévenot et al., 2017). It should also be noticed that changes in pH and ionic strength within particles can lead to swelling and shrinking phenomena. For instance, van der Sman et al. (2020) modelled the swelling of protein gels during static *in vitro* gastric digestion using the Flory-Rehner theory. This has been combined with the Gibbs-Donnan theory in order to include the distribution of ions between the gastric juice and the protein gel. This model gives insight into the charge of proteins at different pH conditions, the swelling kinetics of the protein gel, and ions transport between gel and gastric juice.

The recentness of the literature cited above illustrates that this field of research has become very active, with much decisive progress made in a short amount of time. In a near future, it is therefore expectable that mechanistic models will be available to simulate and predict the digestion kinetics of macronutrients within particles of gels and solid foods.

3.2.3. Interplays between the hydrolysis of macronutrients

Many research teams focus on either starch, lipid, or protein digestion. This is probably one reason why the interdependence of the hydrolysis of individual macronutrients has been relatively ignored until recently. Nevertheless, it is well admitted that the enzymatic hydrolysis of one macronutrient may be affected by the presence of another one. At small scales, it has been shown that proteolysis of dairy proteins is either favored or disfavored when dairy proteins are adsorbed at the oil-water interface in emulsions (Macierzanka et al., 2009), or impeded by the presence of dietary fiber (Howard & Mahoney, 1989). At the microscale, a great variety of interactions take place between starch, lipid, and protein which, among other things, are believed to play a central role in the glycemic response to foods (Parada & Santos, 2016). As already illustrated, the macrostructure of foods also have a large impact on the hydrolysis of macronutrients. Some effects are expectable, as for instance the fact that starch digestion is facilitated by the alveolar structure of bread compared to the compact structure of pasta (Mishra, Hardacre, and Monro 2012), or that a faster protein digestion of Bambara groundnuts is accompanied by a faster digestion of the entrapped starch (Gwala et al., 2020). However, other effects are less obvious, as for instance that a similar, or even higher, final extent of lipolysis may be obtained for an emulsion entrapped in a protein network in comparison with an equivalent protein solution (Mat et al., 2020).

Although they studied unprocessed wheat flour, i.e. not exactly an edible food, Bhattarai, Dhital, and Gidley (2016) performed a thorough investigation of the main enzymatic reactions during static *in vitro* digestion, and reached the following conclusions: (1) the presence of individual intact macronutrients consistently attenuates the enzymatic digestion of other macronutrients, (2) the biopolymer components (starch, protein) interact with each other so that enzymatic digestion of each is impeded, and (3) there are synergistic effects of salivary α-amylase, pepsin and pancreatin on nutrient hydrolysis. It may be assumed that the first two observations of Bhattarai, Dhital, and Gidley (2016) reflect shielding effects from one macronutrient to another. These shielding effects can result from specific interactions between the macronutrients. They may also result from lower meeting probability of the enzyme with its substrate, because their

diffusion are hindered by the presence of other substrates and/or because of an increased surface for non-productive (i.e. non-substrate) binding of enzymes (Dhital et al., 2015; Pluschke et al., 2017). In any case, it is interesting to note that models have been proposed in cases where shielding effects are more obvious, *i.e.* for entrapped macronutrients. A good example thereof is the impact of cell wall encapsulation on the hydrolysis of starch in whole grain foods, which typically shows an initial lag phase for the time needed by α-amylase to diffuse through the cell wall. Similar to what was proposed for non-processed sweet potato (Liu & Sopade, 2011), recent studies by Pallares et al. (2018; 2019) showed that the *in vitro* digestion kinetics of encapsulated starch of cooked common bean cotyledons can be well described by a logistic model. Recently, a more mechanistic model validated on *in vitro* data has also been proposed to hierarchize the physical and kinetic factors involved in the reduction of bean starch hydrolysis (Rovalino-Córdova et al., 2021), highlighting the key effects of enzyme adsorption and diffusion through the cell wall. Interestingly, a comparable approach has been proposed by Sarkar et al. (2016) to model a rate-limiting phase of lipase diffusion for the hydrolysis of Pickering emulsions having a densely packed layer. This illustrates that similar modelling approaches are developed and used for different macronutrients, hence suggesting that a common framework might be suitable for entrapped macronutrients.

The studies cited above clearly support the idea that the enzymatic hydrolysis of starch, lipid, and protein should all be considered simultaneously to improve our understanding of their interplays, and refine our modelling approaches. To the best of the authors' knowledge, no mathematical models have yet been proposed to incorporate an interdependence of enzymatic hydrolysis reactions in the context of the digestion of several macronutrients. This constitutes an interesting area for future research, which calls for more studies combining *in vitro* digestion and mathematical modelling.

4. Towards modelling of nutrient hydrolysis, release, and transit in dynamic *in vitro* conditions

As reviewed elsewhere (Li et al., 2020), various *in vitro* dynamic digestion devices have been developed to study the digestion behavior of food in more physiologically relevant conditions. The INFOGEST consortium has also published recently a semi-dynamic digestion protocol that considers gradual gastric acidification, secretions, and emptying, and that can be set up without sophisticated equipment (Mulet-Cabero et al., 2020). The ongoing standardization and dissemination of these protocols and materials will provide many sets of comparable data, which are difficult to fully interpret without a quantitative dynamic modelling. Indeed, during these experiments, not only the physicochemical conditions (pH, enzyme concentrations) change as a function of time, but the substrates and the hydrolytic products also transit from one compartment to another. *In silico* models describing the process flows of these dynamic digestion experiments can serve as a useful means to better understand and quantify the system dynamics, and hence be of real added value to interpret experimental data. These models can be built based on an engineering approach (Bornhorst et al., 2016; Lamond et al., 2019), where mass balance principles are applied over each reaction vessel (*i.e.* digestive compartment) for each compound of interest:

$$Mass\ of\ compound = Input - Output + Production - Consumption \quad (1)$$

$$Vessel\ volume = Basal\ volume + \ Input - Output \quad (2)$$

In the following sections, we intend to show that this rather simple principles can be combined with previously described enzymatic reaction models, and used to more thoroughly interpret (semi-)dynamic *in vitro* data, make predictions, and even guide the experimental work. For pedagogical purposes, we will first consider the case of a gastric digestion with dynamic secretions but no emptying (i.e. no transit), before discussing the case of multi-compartment dynamic *in vitro* digestion systems.

4.1. *In vitro* gastric digestion with secretion but no emptying

The static conditions (constant pH and enzyme concentration) of intestinal *in vitro* digestion are often considered as relatively close to the physiological reality. This is not the case for the gastric phase, during which the dynamic nature of the HCl and enzyme secretions directly influence the hydrolysis reactions. In this section, we will therefore focus on the case of *in vitro* gastric digestion with gradual secretions of HCl and enzymes, but with no emptying (this will be discussed in section 4.2).

In such an experiment, it is relatively easy to calculate the concentration of enzymes over time in the gastric compartment using the principle described in Eq. 1 and 2. The mass of salivary amylase (if used) is most generally constant since it is added before the beginning of the gastric phase, while pepsin and gastric lipase (if used) incoming fluxes, from stock solutions of known concentrations, are finely controlled. As detailed later on, the evolution of gastric pH is much less trivial to predict from the flux of incoming HCl because of the buffering capacity of the meal. Nevertheless, gastric pH is, in practice, always experimentally monitored when dynamic secretions of HCl are used. Hence, models of macronutrient hydrolysis in dynamic *in vitro* conditions do not have to explicitly include this buffering effect. They can simply use experimental pH data, either directly using data interpolation or indirectly using an empirical equation that fits the corresponding data.

When no food structure effects are expected to occur (*e.g.* a liquid food that does not phase separate), the pH-dependence of the enzyme activities are the only remaining variables. Although the pH-dependence of enzymes may vary from one substrate to another, one can reasonably build upon experimental data reported in the literature in a first approach. Figure 2A provides an example of a data set for all enzymes generally included in an *in vitro* gastric digestion experiment (Brodkorb et al., 2019; Mulet-Cabero et al., 2020). The maximum activity of human salivary amylase is around pH 6-7, and gradually decreases until it is totally inactivated around pH 3.0-3.5 (Bernfeld et al., 1948; Freitas et al., 2018; Freitas & Le Feunteun, 2019). The activity of porcine pepsin is generally negligible for pH above 5 and has its maximum around pH 2 (Kondjoyan et al., 2015; Pletschke et al., 1995). The activity of the lipase from rabbit gastric extract

shows a bell shape with an optimum around pH 4 on long-chain triglycerides (Moreau et al., 1988; Sams et al., 2016).

As shown in Figure 2A, the pH-dependence of enzyme activities can be mathematically modelled from such a data set (see supplementary data for details), and thus be estimated during the course of an *in vitro* experiment providing that the time-evolution of pH is monitored. As an example, Figure 2B shows the predicted activities of human salivary amylase, porcine pepsin, and rabbit gastric lipase as function of time (see supplementary data for details), assuming that gastric pH follows the model proposed by Van Wey et al. (2014) of the human gastric pH data of Malagelada, Go, and Summerskill (1979). This simulation nicely illustrates the chronology of enzymatic actions that can be expected when conducting such a gastric *in vitro* experiment in which pH evolves. It also gives an insight on how mathematical models of enzymatic hydrolysis that are traditionally applied to data obtained in static conditions (e.g. constant pH and enzyme concentrations) could be transposed to cases where dynamic gastric secretions are used, and for which both the evolving pH and enzyme concentration need to be taken into account. In a first approach, one could for instance weight (multiplying pre-factors) the hydrolysis rate constant of the models described so far (Table 2), by making it proportional to: (1) the increasing concentration of the corresponding enzyme, calculated from the incoming fluxes (Eq. 1 and 2), and (2) its activity as function of pH (as exemplified in Fig. 2A) and/or time (as exemplified in Fig. 2B), providing that the decrease in pH over time is experimentally monitored. It should be highlighted, however, that such a task is hardly compatible with an analytical solution. It rather calls for the use of a computer program to numerically solve a system of differential equations. Although more complex for non-initiated people, this does not constitute a real challenge for modellers.

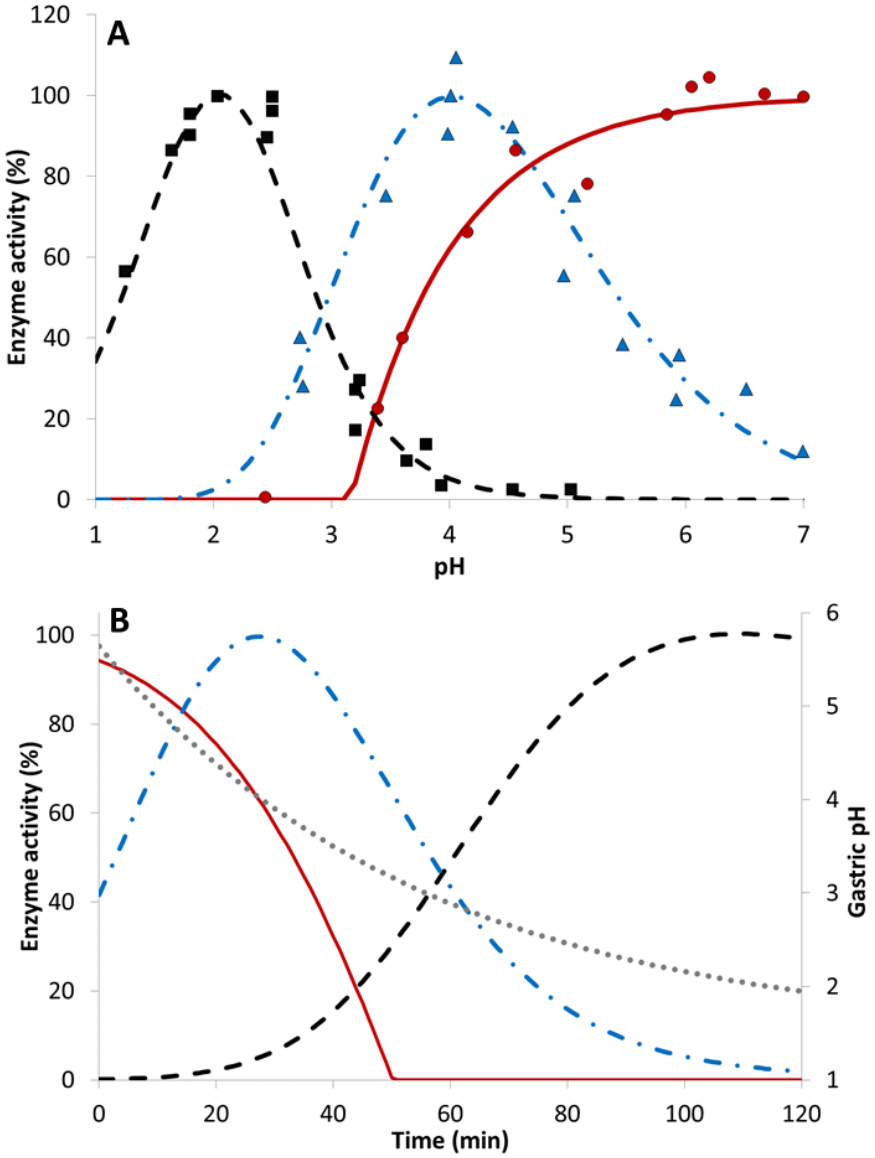

Figure 2*: (A) Activity of human salivary amylase (red circles and solid line), porcine pepsin (black squares and dashed line), and rabbit gastric lipase (blue triangles and dashed-dotted line) as a function of pH. Experimental data (symbols) have been reproduced from* (Freitas et al., 2018) *for human salivary amylase,* (Kondjoyan et al., 2015) *and* (Pletschke et al., 1995) *for porcine pepsin, and* (Moreau et al., 1988) *for the rabbit gastric lipase on long-chain triglycerides. Modelled data (lines) are described in supplementary data. (B) Predicted enzyme activities as a function of time (left hand y-axis, same line style and colour as in Figure 2A) assuming a decreasing gastric pH (grey dotted line, right hand y-axis) that follows the model proposed by* Van Wey et al. (2014) *of the human gastric pH data of* Malagelada, Go, and Summerskill (1979).

It is noteworthy that gastric *in vitro* digestion data obtained with dynamic secretions also offer a real opportunity to improve our capability to model some of the key mechanisms that are partly hidden in static *in vitro* conditions, but that do take place during *in vivo* gastric digestion. These notably include our capability to predict the gastric pH from the flux of HCl and the food buffering properties, the effects of a more reliable chronology of enzymatic reactions (Figure 2B), of solid food particle size, and of their swelling/shrinking as a function of pH. In particular, the buffering capacity of foods is a key factor to consider in (semi-)dynamic gastric conditions because it largely governs the evolution of pH, and hence, of enzymatic activities. Although a regression model of the buffering capacity of foods accounting for the protein content of foods, the protein type, and the surface area of particles for solid foods has been proposed (Mennah-Govela et al., 2019), a more general model accounting for the flux of secretions still need to be developed. In a recent study performed by Sicard et al. (2018) on the modelling of a semi-dynamic gastric digestion of beef meat, the parameters used to predict the variations of the buffering capacity during HCl penetration were determined from *in vitro* experiments. The importance of considering the meat buffering capacity to correctly predict the digestion of meat proteins is illustrated in Figure 3. When the model ignores the buffering capacity of 4.25 mm-sized meat particles and assumes a constant gastric pH of 1.5 (red solid line), the pH of meat particles drops below 2.0 in less than 5 min, whereas it takes over 55 min when the buffering effect is included in the simulation (blue dashed line). Moreover, the model can also be used to predict the pH inside the meat particles in more realistic pH conditions, *i.e.* with a progressive acidification of the gastric content. Using the same human-based pH acidification curve as in Figure 2 (grey dotted line), one clearly sees that the internal pH of meat particles decreases very slowly (green dashed-dotted line). According to model predictions, it will take more than 1.5 h to reach pH 2.9 (Figure 3), for which pepsin reaches 50% of its maximal activity (Figure 2A). This illustrates how considerable the impact of food buffering on protein hydrolysis by pepsin can be in the case of meat particles of several millimetres in size.

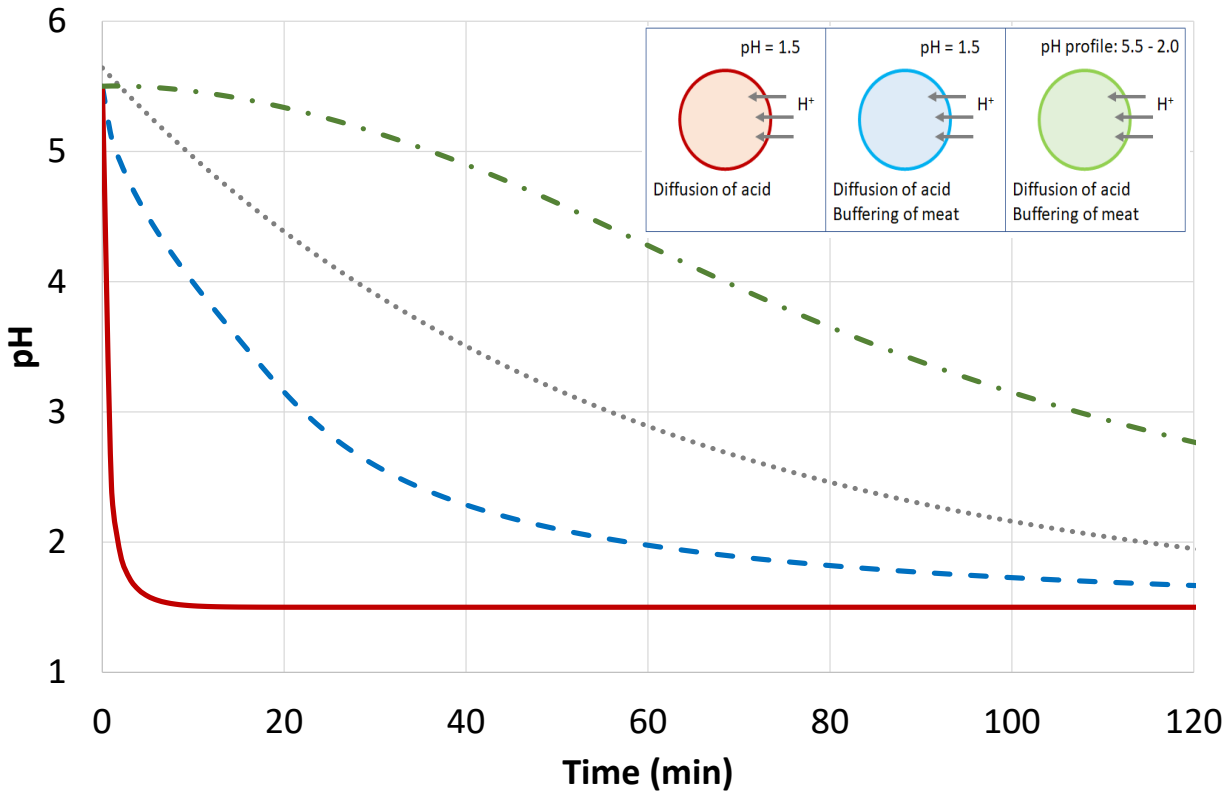

***Figure 3**: Average pH in a meat particle of 4.25 mm-diameter calculated with the model of* Sicard et al. (2018) *during gastric digestion. Simulations were performed for: (1) a constant gastric pH of 1.5 with no buffering capacity of meat (red solid line), (2) a constant gastric pH of 1.5 with consideration of the buffering capacity of meat (blue dashed line), and (3) assuming the same decreasing gastric pH as in Figure 2B (based on human data) with consideration of the buffering capacity of meat. Main assumptions in these simulations are that the migration of protons can be assimilated to Fickian diffusion and that the local variations of pH can be described by equations validated separately from experiments conducted in vitro to determine the buffering capacity of the meat. Readers interested in knowing more about this model, which also include protein hydrolysis by pepsin, and its underlying assumptions, are kindly referred to the quoted paper.*

It should be noticed that the model proposed by Sicard et al. (2018) did not simply intend to predict the pH evolution inside meat particles. They built a reaction-diffusion model that accounts for other mechanisms besides proton diffusion and meat buffering capacity: pepsin diffusion in bolus particles, the pH-dependence of pepsin activity, as well as the gastric fluid velocity. This

model still has room for improvement, in particular by computing the progressive reduction of the particle size in relation to gastric emptying kinetics. Such an approach could also be combined with the model proposed by van der Sman et al. (2020) on the swelling of protein gel particles, which accounts for gel properties, gastric pH evolution, as well as the ion transport in and out of the particles.

These pioneer works on the modelling of *in vitro* gastric digestion that consider semi-dynamic conditions are very promising. They should enable further modelling developments on the digestion of solid particles to advance beyond enzymatic hydrolysis models that consider steady-state physicochemical conditions. To a certain extent, they also appear capable of accounting for the effects of the mechanical movements and of the food structure on the hydrolysis of macronutrients in more physiologically relevant *in vitro* conditions. However, much more experimental and modelling efforts are needed before such developments can give birth to a model capable of simulating the dynamics of the main phenomena taking place during gastric digestion. To give a sample of the facing challenges, we may point to the current lack of models to simultaneously consider the three main enzymatic reactions and their interplays; to mechanistically simulate the interrelations between the buffering capacity, the food composition, and the particle sizes; to predict mixing of food with the secretions; or to compute the reduction of particle size upon hydrolysis.

4.2. Dynamic multicompartment *in vitro* gastrointestinal digestion

This section considers the case of dynamic *in vitro* digestion with both incoming and emptying fluxes. These experiments are most generally performed with computer-controlled devices such as the TNO gastrointestinal model (TIM-1) (Minekus et al., 1995), the Human Gastric Simulator (HGS) (Kong & Singh, 2010), or the DIDGI system (Ménard et al., 2014) to name a few (Li et al., 2020). In these more complex experiments, both the incoming and emptying fluxes arriving and leaving one *in vitro* digestion compartment have to be taken into account in the mass balance equations. Considering the case of one substrate and one hydrolysis product in the gastric vessel of a dynamic *in vitro* digestion system, the mass balance equations may be written as:

$$\frac{dV_G}{dt} = \emptyset_{IN} + \emptyset_{GS} - \emptyset_{GE} \quad (3)$$

$$\frac{dm_G^S}{dt} = \emptyset_{IN} \times C_{meal}^S - \emptyset_{GE} \times C_{GE}^S - R_H \quad (4)$$

$$\frac{dm_G^P}{dt} = R_H - \emptyset_{GE} \times C_{GE}^P \quad (5)$$

with V_G the volume of gastric content, $\emptyset_{IN}$ the flux of meal ingestion (if any), $\emptyset_{GS}$ the flux of gastric secretions, and $\emptyset_{GE}$ the flux of gastric emptying. m_G^S and m_G^P are the masses of substrate and product in the gastric compartment, respectively, C_{meal}^S is the substrate concentration in the meal, C_{GE}^S and C_{GE}^P are the substrate and product concentrations in the material that is being emptied from the gastric compartment. Finally, R_H represents the rate of hydrolysis (in a unit of mass per unit of time, in our example) that converts the substrate *S* into the product *P*, *i.e.* the mathematical model of enzymatic hydrolysis.

A similar set of equations could be written for the intestinal compartment, and as described in the next sections, these can be numerically solved (providing that some data are experimentally measured) to enable the volumes, the masses and the concentrations (since *C = m/V*) of the considered species to be calculated as a function of time. In the following, we intend to show that such a modelling approach can be very useful to interpret dynamic *in vitro* digestion data, and may even guide the experimental work. We will first consider the ideal case of homogeneous liquid meals, for which the transit can be accurately simulated, before considering cases where food transit becomes very difficult or impossible to predict.

4.2.1. The ideal case: Homogenous solutions

For homogeneous liquid meals that mix well with the digestive fluids, we may assume the *in vitro* compartments to behave as perfectly stirred reactors. Under such circumstances, C_{GE}^S and C_{GE}^P, the substrate and product concentrations of the matter leaving the stomach directly correspond to their respective concentrations in the gastric compartment. Providing that these can be experimentally determined, that the substrate concentration in the meal (C_{meal}^S) is known, and that all the fluxes ($\emptyset$) are known in dynamic *in vitro* experiments, the only remaining unknowns

lie within the mathematical model of the enzymatic hydrolysis (R_H) that is used (Eq. 4 and 5). In this ideal case, it is thus possible to retrieve enzymatic hydrolysis parameter(s) by fitting dynamic *in vitro* digestion data with such a set of equations.

The transit of a homogenous solution during dynamic *in vitro* digestion can thus be adequately predicted from the experimental conditions. The main modelling challenge is to find a suitable model of the enzymatic hydrolysis reaction that accounts for the evolving pH (providing it is monitored) and enzyme concentration. Although more work is still needed in that area, as discussed in section 4.1., this does not appear as an insurmountable task. With a homogenous solution, all main phenomena (i.e. hydrolysis and transit) taking place can thus be theoretically modelled, hence providing a general picture of the whole experiment. Indeed, with a model capable of simulating the experimental process flow, one could simultaneously evaluate the quantities of substrate and product that enter and leave a digestive compartment (*e.g.* stomach or small intestine), that are consumed/produced by hydrolysis, and calculate the extent of dilution induced by the digestive secretions. In fact, for homogenous solutions, all conditions seem met to start building digital twins of dynamic *in vitro* systems in order to simulate different scenarios (more or less enzymes, larger or smaller emptying fluxes, *etc*.), refine experimental *in vitro* protocols (*e.g.* by enabling rapid comparisons of predicted outcomes with the *in vivo* literature), or serve as an alternative to some experiments.

4.2.2. The general case: Heterogeneous complex foods or meals

In practice, most of the foods we eat are solid, semi-solid, or unstable liquids. As discussed in section 3.2.1, even when they initially are homogenous solutions, the physicochemical conditions encountered within the GI tract may lead to structural heterogeneities and alter the kinetics hydrolysis. During dynamic *in vitro* digestions, these can also largely impact the kinetics of food transit (Mulet-Cabero et al., 2019). It would be really difficult to develop a mathematical model capable of reproducing heterogeneity phenomena as well as their impact on macronutrient hydrolysis and transit. We may nevertheless argue that the system of equations presented in the previous section can still be useful to gain insights on the behaviour of complex foods, and/or help to optimize the sampling strategy.

For instance, let us consider the case of an oil-in-water emulsion that tends to cream in the gastric and intestinal compartments of a dynamic digestion system with one gastric and one intestinal compartment. As the creaming progresses, the material that is emptied from the bottom of the stomach will be less concentrated than expected from simple dilution considerations. In this example, it becomes highly questionable to rely on sample analysis to monitor the creaming and the lipolysis reaction(s) because the amount and type of lipid collected will clearly depend on both the sampling time and the exact position of the sample collection. From the authors' knowledge, most research teams working on the digestion of emulsions in dynamic conditions have already faced this issue. The 'one experience per time point' strategy sometimes used in static *in vitro* conditions to overcome such problems (Egger et al., 2019; Giang et al., 2015, 2016), which consists in stopping the experiment after different times to collect and analyse all the gastric and/or intestinal contents, is a suitable option. However, it is far from being time and cost efficient in dynamic conditions. From a modelling point of view, the oil concentration in the gastric emptying flux (C_{GE}^{S} in Eq. 4) is the only variable needed to recalculate the oil fraction (1) that is retained in the gastric compartment versus (2) that is transiting to the small intestine. It follows that one can more simply reconstruct the transit of the oil by analysing samples that are collected from the transit tubing (*i.e.* at the stomach and small intestinal exits in our example), using data interpolation or regression to numerically solve the model. Such a sampling strategy combined with mathematical modelling can indeed enable the kinetics of both oil creaming and the lipolysis reaction(s) to be estimated altogether.

This reasoning not only holds for oil creaming but also for all phenomena that may alter the *in vitro* transit of a compound (aggregation, gastric retention of large particles, *etc*.). The kinetics of transit can always be estimated *a posteriori* from the analysis of the transiting matter and an adequate modelling approach. When combined with dynamic *in vitro* digestion experiments, modelling may therefore not only be used as a tool to fit experimental data or simulate potential scenarios, but also to quantitatively investigate the effects of food structure (or re-structuring) on the observed transit and hydrolysis, providing that the sampling strategy has been implemented accordingly.

5. From lessons learned *in vitro* to the *in vivo* world: Prospects on future uses of these mathematical models

Preceding sections have illustrated that mathematical modelling and *in vitro* digestion approaches are highly complementary methods. Mathematical models can be used to recover a full picture of the phenomena taking place during static up to dynamic *in vitro* digestion experiments, meanwhile *in vitro* procedures offer an excellent framework to test and improve our modelling capabilities of poorly understood mechanisms. Beyond these considerations, mathematical modelling of the digestive processes may also offer a decisive means to bridge the gap between *in vitro* and *in vivo* observations.

Despite their undeniable usefulness and success, *in vitro* methods cannot capture all the intricacies of the digestive process. This is why a number of concerns need to be addressed to translate *in vitro* findings into human and animal nutritional benefits or risks. Our knowledge of certain biological processes that are not captured by *in vitro* experiments (e.g. feedback mechanisms) can nonetheless be modelled mathematically. Thus, hybrid research strategies can be built by combining *in vitro* measurements and *in silico* extrapolations to better predict possible *in vivo* consequences. For instance, it has been shown that mathematically estimated human blood glucose disposal rates can be subtracted from starch hydrolysis profiles obtained under static conditions to reproduce the bell shape of *in vivo* glycemic responses (Monro, Mishra, and Venn 2010). Recently, Bellmann et al. (2018) could even accurately predict the human glycemic responses to 22 different foods by combining dynamic *in vitro* experiments and an *in silico* approach integrating a glucose-insulin homeostatic model. In the future, it is thus expectable that other *in vitro-in silico* strategies will be proposed to better project *in vitro* findings (e.g. nutrient bioaccessibility) into *in vivo* outcomes (e.g. nutrient bioavailability).

Mathematical models based on *in vitro* digestion data can also be very useful to improve *in silico* representations of the functioning of the GI tract. Most of the models built on an *in vitro* approach focus on mechanisms that can hardly be studied *in vivo*, such as the effects of food particle size, microstructure, and buffering capacity on macronutrient digestion kinetics. Therefore, *in vitro*

based mathematical models such as those presented in this review will certainly enable to improve and refine *in silico* models of *in vivo* digestion (Le Feunteun et al., 2020, 2021). It should indeed be understood that the complexity of modelling the digestive process is not only due to the large number of different molecules, enzymes, and biological processes involved. As illustrated in Figure 4, the length scales involved in these processes are also very different (Bornhorst et al., 2016). As an example, enzymatic reactions take place at the molecular scale. Substrates, especially in a structured food matrix, are organized at the microscale, while bolus formation, peristalsis and gastric emptying take place at the macroscale. Thus, digestion rates depend strongly on the interactions between multiple processes that take place at different length and time scales. Mathematical modelling, by using parameters obtained from *in vivo* and *in vitro* measurements, allows a better understanding of these interactions. Therefore, they directly contribute to our understanding of the digestion of foods, and may help to advance towards a more complete *in silico* representation of the digestion process.

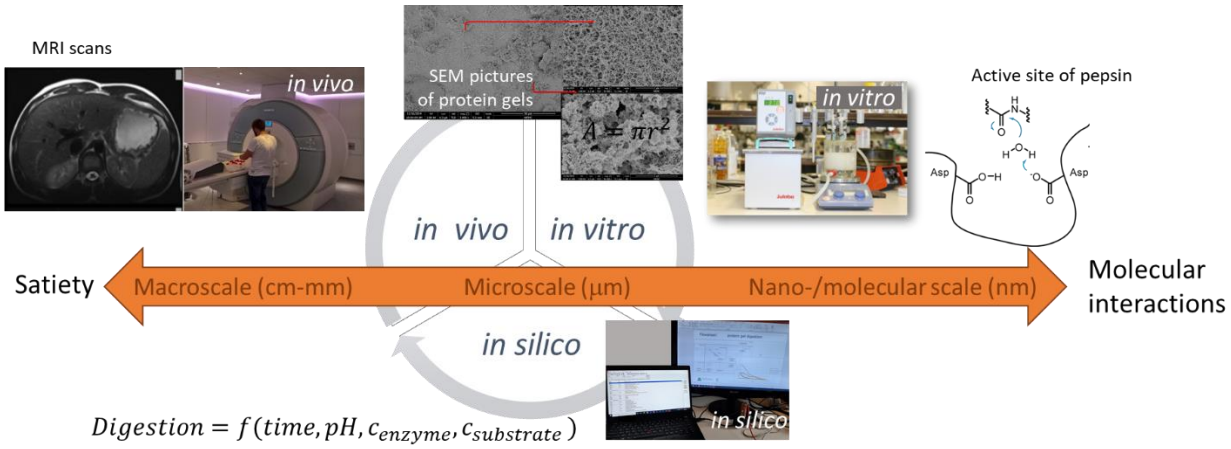

Figure 4*: Multiple length scales are involved in the digestion process. At small scales, numerous molecular interactions take place. At microscale, the inner structure of the foods may limit the diffusion of acid/base and enzyme, or entrap macronutrients. The gastrointestinal motility, food transport and solid particle breakdown occur at the macroscale.*

In vivo experiments and observational studies will undoubtedly remain the gold standard to assess the health effect of a food. However, considering that experiments involving humans or animals tend to be more and more restricted by ethical concerns, both *in vitro* and *in silico*

methods seem destined to a bright future. By providing a common language between *in vitro* and *in vivo* observations, mathematical modelling can help anticipating the *in vivo* consequences of *in vitro* findings, and *vice versa*, be useful to improve *in vitro* methods and data interpretation. Although this area of research remains in its early years, it is clear that further developments in mathematical modelling and *in vitro-in silico* hybrid strategies will play an important role in the future of food and nutrition sciences. For this, we need to target a food scientist generation not refraining from using mathematical modelling approaches through sufficient training during their educational background.

6. Conclusion

An important factor in the translation of the digestion of single macronutrients to the digestion of a whole food, is that the digestion of a macronutrient is often influenced by the presence of other ones or other minor components that are present in the food. To date, most modelling efforts have focused on static *in vitro* digestion of single macronutrients (starch, lipid, protein). In reality, however, foods are not neat substrates, but are highly structured systems formed by a combination of macronutrients that are usually not homogeneously distributed within the food. Another level of complexity is related to the multiscale structures in the foods and their evolution, with a determinant impact of solid particle size and/or of phase separation phenomena. This is particularly important in dynamic *in vitro* conditions, during which the gradual addition of digestive fluids may largely modify food structures.

There is currently an increasing number of mathematical models developed that consider (1) the effect of solid food particle size, the food buffering capacity, food microstructure, *etc*. and (2) the dynamic nature of the digestion processes. Although much of the added value that can be obtained by coupling *in silico* approaches to *in vitro* experimentation remains to be exploited, the authors strongly believe that this marriage can lead to significant progresses in both *in silico* and *in vitro* prediction capabilities.

Acknowledgments

The authors would like to acknowledge INFOGEST COST Action FA1005 (2011–2015) and subsequently the INFOGEST network funded by INRAE for enabling the networking required to write this review. The authors acknowledge the company “Zucker & Jagdwurst” for their permission to use one of their photo to illustrate complex foods in Fig. 1. S.H.E. Verkempinck is a Postdoctoral Researcher funded by the Research Foundation Flanders (FWO - Grant no. 1222420N).

Conflict of interest

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

CRediT roles

Steven Le Feunteun: Conceptualization, Visualization, Writing - original draft
Sarah Verkempinck: Visualization, Writing - original draft
Juliane Floury: Writing - original draft
Anja Janssen: Visualization, Writing - original draft
Alain Kondjoyan: Writing - original draft
Sebastien Marze: Writing - original draft
Pierre-Sylvain Mirade: Writing - original draft
Anton Pluschke: Writing - original draft
Jason Sicard: Visualization, Writing - original draft
George van Aken: Writing - original draft
Tara Grauwet: Conceptualization, Visualization, Writing - original draft

References

Bellmann, S., Minekus, M., Sanders, P., Bosgra, S., & Havenaar, R. (2018). Human glycemic response curves after intake of carbohydrate foods are accurately predicted by combining in vitro gastrointestinal digestion with in silico kinetic modeling. *Clinical Nutrition Experimental*, *17*, 8–22. https://doi.org/10.1016/j.yclnex.2017.10.003

Bernfeld, P., Staub, A., & Fischer, Ed. H. (1948). Sur les enzymes amylolytiques XI. Propriétés de l'α-amylase de salive humaine cristallisée. *Helvetica Chimica Acta*, *31*(7), 2165–2172. https://doi.org/10.1002/hlca.19480310738

Besselink, T., Baks, T., Janssen, A. E. M., & Boom, R. M. (2008). A stochastic model for predicting dextrose equivalent and saccharide composition during hydrolysis of starch by α-amylase. *Biotechnology and Bioengineering*, *100*(4), 684–697. https://doi.org/10.1002/bit.21799

Bhattarai, R. R., Dhital, S., & Gidley, M. J. (2016). Interactions among macronutrients in wheat flour determine their enzymic susceptibility. *Food Hydrocolloids*, *61*, 415–425. https://doi.org/10.1016/j.foodhyd.2016.05.026

Bohn, T., Carriere, F., Day, L., Deglaire, A., Egger, L., Freitas, D., Golding, M., Le Feunteun, S., Macierzanka, A., Menard, O., Miralles, B., Moscovici, A., Portmann, R., Recio, I., Rémond, D., Santé-Lhoutelier, V., Wooster, T. J., Lesmes, U., Mackie, A. R., & Dupont, D. (2018). Correlation between in vitro and in vivo data on food digestion. What can we predict with static in vitro digestion models? *Critical Reviews in Food Science and Nutrition*, *58*(13), 2239–2261. https://doi.org/10.1080/10408398.2017.1315362

Bornhorst, G. M., Gouseti, O., Wickham, M. S. J., & Bakalis, S. (2016). Engineering Digestion: Multiscale Processes of Food Digestion. *Journal of Food Science*, *81*(3), R534–R543. https://doi.org/10.1111/1750-3841.13216

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R., Carrière, F., Clemente, A., Corredig, M., Dupont, D., Dufour, C., Edwards, C., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., … Recio, I. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols*, *14*(4), 991–1014. https://doi.org/10.1038/s41596-018-0119-1

Butterworth, P. J., Warren, F. J., Grassby, T., Patel, H., & Ellis, P. R. (2012). Analysis of starch amylolysis using plots for first-order kinetics. *Carbohydrate Polymers*, *87*(3), 2189–2197. https://doi.org/10.1016/j.carbpol.2011.10.048

Capuano, E., & Janssen, A. E. M. (2021). Food matrix and macronutrient digestion. *Annual Review of Food Science and Technology*, *12*(1), 11.1-11.20. https://doi.org/10.1146/annurev-food-032519-051646

Carriere, F., Barrowman, J. A., Verger, R., & René, L. (1993). Secretion and contribution to lipolysis of gastric and pancreatic lipases during a test meal in humans. *Gastroenterology*, *105*(3), 876–888. https://doi.org/10.1016/0016-5085(93)90908-U

Cornish-Bowden, A. (2015). One hundred years of Michaelis–Menten kinetics. *Perspectives in Science*, *4*, 3–9. https://doi.org/10.1016/j.pisc.2014.12.002

Day, L., Golding, M., Xu, M., Keogh, J., Clifton, P., & Wooster, T. J. (2014). Tailoring the digestion of structured emulsions using mixed monoglyceride-caseinate interfaces. *Food Hydrocolloids*, *36*, 151–161. https://doi.org/10.1016/j.foodhyd.2013.09.019

Deng, Y., van der Veer, F., Sforza, S., Gruppen, H., & Wierenga, P. A. (2018). Towards predicting protein hydrolysis by bovine trypsin. *Process Biochemistry*, *65*, 81–92. https://doi.org/10.1016/j.procbio.2017.11.006

Dhital, S., Gidley, M. J., & Warren, F. J. (2015). Inhibition of α-amylase activity by cellulose: Kinetic analysis and nutritional implications. *Carbohydrate Polymers*, *123*, 305–312. https://doi.org/10.1016/j.carbpol.2015.01.039

Dhital, S., Warren, F. J., Butterworth, P. J., Ellis, P. R., & Gidley, M. J. (2017). Mechanisms of starch digestion by α-amylase-Structural basis for kinetic properties. *Critical Reviews in Food Science and Nutrition*, *57*(5), 875–892. https://doi.org/10.1080/10408398.2014.922043

Dona, A. C., Pages, G., Gilbert, R. G., & Kuchel, P. W. (2010). Digestion of starch: In vivo and in vitro kinetic models used to characterise oligosaccharide or glucose release. *Carbohydrate Polymers*, *80*(3), 599–617. https://doi.org/10.1016/j.carbpol.2010.01.002

Dupont, D., Le Feunteun, S., Marze, S., & Souchon, I. (2018). Structuring food to control its disintegration in the gastrointestinal tract and optimize nutrient bioavailability. *Innovative Food Science & Emerging Technologies*, *46*, 83–90. https://doi.org/10.1016/j.ifset.2017.10.005

Edwards, C. H., Warren, F. J., Milligan, P. J., Butterworth, P. J., & Ellis, P. R. (2014). A novel method for classifying starch digestion by modelling the amylolysis of plant foods using first-order enzyme kinetic principles. *Food Funct.*, *5*(11), 2751–2758. https://doi.org/10.1039/C4FO00115J

Egger, L., Ménard, O., Baumann, C., Duerr, D., Schlegel, P., Stoll, P., Vergères, G., Dupont, D., & Portmann, R. (2019). Digestion of milk proteins: Comparing static and dynamic in vitro

digestion systems with in vivo data. *Food Research International*, *118*, 32–39. https://doi.org/10.1016/j.foodres.2017.12.049

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, *46 Suppl 2*, S33-50.

Floury, J., Bianchi, T., Thévenot, J., Dupont, D., Jamme, F., Lutton, E., Panouillé, M., Boué, F., & Le Feunteun, S. (2018). Exploring the breakdown of dairy protein gels during in vitro gastric digestion using time-lapse synchrotron deep-UV fluorescence microscopy. *Food Chemistry*, *239*, 898–910. https://doi.org/10.1016/j.foodchem.2017.07.023

Freitas, D., & Le Feunteun, S. (2019). Inhibitory effect of black tea, lemon juice, and other beverages on salivary and pancreatic amylases: What impact on bread starch digestion? A dynamic in vitro study. *Food Chemistry*, *297*, 124885. https://doi.org/10.1016/j.foodchem.2019.05.159

Freitas, D., Le Feunteun, S., Panouillé, M., & Souchon, I. (2018). The important role of salivary α-amylase in the gastric digestion of wheat bread starch. *Food & Function*, *9*(1), 200–208. https://doi.org/10.1039/C7FO01484H

Gaucel, S., Trelea, I. C., & Le Feunteun, S. (2015). Comment on New mathematical model for interpreting pH-stat digestion profiles: Impact of lipid droplet characteristics on in vitro digestibility. *Journal of Agricultural and Food Chemistry*, *63*(47), 10352–10353. https://doi.org/10.1021/acs.jafc.5b03573

Giang, T. M., Gaucel, S., Brestaz, P., Anton, M., Meynier, A., Trelea, I. C., & Le Feunteun, S. (2016). Dynamic modeling of in vitro lipid digestion: Individual fatty acid release and

bioaccessibility kinetics. *Food Chemistry*, *194*, 1180–1188. https://doi.org/10.1016/j.foodchem.2015.08.125

Giang, T. M., Le Feunteun, S., Gaucel, S., Brestaz, P., Anton, M., Meynier, A., & Trelea, I. C. (2015). Dynamic modeling highlights the major impact of droplet coalescence on the in vitro digestion kinetics of a whey protein stabilized submicron emulsion. *Food Hydrocolloids*, *43*, 66–72. https://doi.org/10.1016/j.foodhyd.2014.04.037

Golding, M., Wooster, T. J., Day, L., Xu, M., Lundin, L., Keogh, J., & Clifton, P. (2011). Impact of gastric structuring on the lipolysis of emulsified lipids. *Soft Matter*, *7*(7), 3513. https://doi.org/10.1039/c0sm01227k

Goñi, I., Garcia-Alonso, A., & Saura-Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research*, *17*(3), 427–437.

Gwala, S., Pallares Pallares, A., Pälchen, K., Hendrickx, M., & Grauwet, T. (2020). In vitro starch and protein digestion kinetics of cooked Bambara groundnuts depend on processing intensity and hardness sorting. *Food Research International*, *137*, 109512. https://doi.org/10.1016/j.foodres.2020.109512

Gwala, S., Wainana, I., Pallares Pallares, A., Kyomugasho, C., Hendrickx, M., & Grauwet, T. (2019). Texture and interlinked post-process microstructures determine the in vitro starch digestibility of Bambara groundnuts with distinct hard-to-cook levels. *Food Research International*, *120*, 1–11. https://doi.org/10.1016/j.foodres.2019.02.022

Herman, R., Gao, Y., & Storer, N. (2006). Acid-induced unfolding kinetics in simulated gastric digestion of proteins. *Regulatory Toxicology and Pharmacology*, *46*(1), 93–99. https://doi.org/10.1016/j.yrtph.2006.05.010

Hoebler, C., Karinthi, A., Devaux, M. F., Guillon, F., Gallant, D. J., Bouchet, B., Melegari, C., & Barry, J. L. (1998). Physical and chemical transformations of cereal food during oral digestion in human subjects. *The British Journal of Nutrition*, *80*(5), 429–436. https://doi.org/10.1017/s0007114598001494

Howard, P., & Mahoney, R. R. (1989). Effect of dietary fibers on the enzymatic digestion of casein. *Food Chemistry*, *34*(2), 141–146. https://doi.org/10.1016/0308-8146(89)90082-4

Infantes-Garcia, M. R., Verkempinck, S. H. E., Guevara-Zambrano, J. M., Andreoletti, C., Hendrickx, M. E., & Grauwet, T. (2020). Enzymatic and chemical conversions taking place during in vitro gastric lipid digestion: The effect of emulsion droplet size behavior. *Food Chemistry*, *326*, 126895. https://doi.org/10.1016/j.foodchem.2020.126895

Kondjoyan, A., Daudin, J.-D., & Santé-Lhoutellier, V. (2015). Modelling of pepsin digestibility of myofibrillar proteins and of variations due to heating. *Food Chemistry*, *172*, 265–271. https://doi.org/ham

Kong, F., & Singh, R. P. (2010). A human gastric simulator (HGS) to study food digestion in human stomach. *Journal of Food Science*, *75*(9), E627-635. https://doi.org/10.1111/j.1750-3841.2010.01856.x

Lamond, A. R., Janssen, A. E. M., Mackie, A., Bornhorst, G. M., Bakalis, S., & Gouseti, O. (2019). An engineering perspective on human digestion. In O. Gouseti, G. M. Bornhorst, S. Bakalis, & A. Mackie (Eds.), *Interdisciplinary Approaches to Food Digestion* (pp. 255–273). Springer International Publishing. https://doi.org/10.1007/978-3-030-03901-1_12

Le Feunteun, S., Al-Razaz, A., Dekker, M., George, E., Laroche, B., & van Aken, G. (2021). Physiologically based modeling of food digestion and intestinal microbiota: State of the

art and future challenges. An INFOGEST Review. *Annual Review of Food Science and Technology*, *12*(1), 16.1-16.19. https://doi.org/10.1146/annurev-food-070620-124140

Le Feunteun, S., Mackie, A. R., & Dupont, D. (2020). In silico trials of food digestion and absorption: How far are we? *Current Opinion in Food Science*, *31*, 121–125. https://doi.org/10.1016/j.cofs.2020.04.006

Li, C., Yu, W., Wu, P., & Chen, X. D. (2020). Current in vitro digestion systems for understanding food digestion in human upper gastrointestinal tract. *Trends in Food Science & Technology*, *96*, 114–126. https://doi.org/10.1016/j.tifs.2019.12.015

Li, H., Dhital, S., Gidley, M. J., & Gilbert, R. G. (2019). A more general approach to fitting digestion kinetics of starch in food. *Carbohydrate Polymers*, *225*, 115244. https://doi.org/10.1016/j.carbpol.2019.115244

Li, Y., & McClements, D. J. (2010). Ma. *Journal of Agricultural and Food Chemistry*, *58*, 8085–8092.

Liu, Y., & Sopade, P. A. (2011). Modelling starch digestion in sweetpotato with biphasic digestograms. *Journal of Food Engineering*, *104*(2), 307–315. https://doi.org/10.1016/j.jfoodeng.2010.12.022

Luo, Q., Borst, J. W., Westphal, A. H., Boom, R. M., & Janssen, A. E. M. (2017). Pepsin diffusivity in whey protein gels and its effect on gastric digestion. *Food Hydrocolloids*, *66*, 318–325. https://doi.org/10.1016/j.foodhyd.2016.11.046

Luo, Q., Chen, D., Boom, R. M., & Janssen, A. E. M. (2018a). Revisiting the enzymatic kinetics of pepsin using isothermal titration calorimetry. *Food Chemistry*, *268*, 94–100. https://doi.org/10.1016/j.foodchem.2018.06.042

Luo, Q., Chen, D., Boom, R. M., & Janssen, A. E. M. (2018b). Revisiting the enzymatic kinetics of pepsin using isothermal titration calorimetry. *Food Chemistry*, *268*, 94–100. https://doi.org/10.1016/j.foodchem.2018.06.042

Luo, Q., Zhan, W., Boom, R. M., & Janssen, A. E. M. (2018). Interactions between acid and proteins under in vitro gastric condition – a theoretical and experimental quantification. *Food & Function*, *9*(10), 5283–5289. https://doi.org/10.1039/C8FO01033A

Lykidis, A., Mougios, V., & Arzoglou, P. (1995). Kinetics of the two-step hydrolysis of triacylglycerol by pancreatic lipases. *European Journal of Biochemistry*, *230*(3), 892–898. Scopus. https://doi.org/10.1111/j.1432-1033.1995.0892g.x

Macierzanka, A., Sancho, A. I., Mills, E. N. C., Rigby, N. M., & Mackie, A. R. (2009). Emulsification alters simulated gastrointestinal proteolysis of β-casein and β-lactoglobulin. *Soft Matter*, *5*(3), 538–550. https://doi.org/10.1039/B811233A

Mahasukhonthachat, K., Sopade, P. A., & Gidley, M. J. (2010). Kinetics of starch digestion in sorghum as affected by particle size. *Journal of Food Engineering*, *96*(1). https://agris.fao.org/agris-search/search.do?recordID=US201301679893

Malagelada, J.-R., Go, V. L. W., & Summerskill, W. H. J. (1979). Different gastric, pancreatic, and biliary responses to solid-liquid or homogenized meals. *Digestive Diseases and Sciences*, *24*, 101–110. https://doi.org/10.1007/BF01324736

Margot, A., Flaschel, E., & Renken, A. (1997). Empirical kinetic models for tryptic whey-protein hydrolysis. *Process Biochemistry*, *32*(3), 217–223. https://doi.org/10.1016/S0032-9592(96)00060-X

Marze, S. (2013). L. *Critical Reviews in Food Science and Nutrition*, *53*(1), 76–108. https://doi.org/10.1080/10408398.2010.525331

Marze, S. (2014). A coarse-grained simulation to study the digestion and bioaccessibility of lipophilic nutrients and micronutrients in emulsion. *Food & Function*, *5*(1), 129–139. https://doi.org/10.1039/c3fo60184f

Marze, S. (2015). Refining in silico simulation to study digestion parameters affecting the bioaccessibility of lipophilic nutrients and micronutrients. *Food & Function*, *6*(1), 115–124. https://doi.org/10.1039/c4fo00483c

Marze, S., & Choimet, M. (2012). In vitro digestion of emulsions: Mechanistic and experimental models. *Soft Matter*, *8*(42), 10982–10993. https://doi.org/10.1039/C2SM26336J

Mat, D. J. L., Souchon, I., Michon, C., & Le Feunteun, S. (2020). Gastro-intestinal in vitro digestions of protein emulsions monitored by pH-stat: Influence of structural properties and interplay between proteolysis and lipolysis. *Food Chemistry*, *311*, 125946. https://doi.org/10.1016/j.foodchem.2019.125946

Maximova, K., & Trylska, J. (2015). Kinetics of trypsin-catalyzed hydrolysis determined by isothermal titration calorimetry. *Analytical Biochemistry*, *486*, 24–34. https://doi.org/10.1016/j.ab.2015.06.027

Ménard, O., Cattenoz, T., Guillemin, H., Souchon, I., Deglaire, A., Dupont, D., & Picque, D. (2014). Validation of a new in vitro dynamic system to simulate infant digestion. *Food Chemistry*, *145*, 1039–1045. https://doi.org/10.1016/j.foodchem.2013.09.036

Mennah-Govela, Y. A., Bornhorst, G. M., & Singh, R. P. (2015). Acid diffusion into rice boluses is influenced by rice type, variety, and presence of α-amylase. *Journal of Food Science*, *80*(2), E316–E325. https://doi.org/10.1111/1750-3841.12750

Mennah-Govela, Y. A., Keppler, S., Januzzi-Guerreiro, F., Follador-Lemos, C., Vilpont, K., & Bornhorst, G. M. (2020). Acid and moisture uptake into red beets during in vitro gastric digestion as influenced by gastric pH. *Food Biophysics*, *15*, 261–272.

Mennah-Govela, Y. A., Singh, R. P., & Bornhorst, G. M. (2019). Buffering capacity of protein-based model food systems in the context of gastric digestion. *Food & Function*, *10*(9), 6074–6087. https://doi.org/10.1039/C9FO01160A

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, null C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., … Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food—An international consensus. *Food & Function*, *5*, 1113–1124. https://doi.org/10.1039/c3fo60702j

Minekus, M., Marteau, P., Havenaar, R., & Veld, J. H. J. Huis in't Veld (1995). A multicompartmental dynamic computer-controlled model simulating the stomach and small intestine. *Alternatives to Laboratory Animals*, *2*, 197–209. https://doi.org/10.1177/026119299502300205

Mishra, S., Hardacre, A., & Monro, J. (2012). Food Structure and Carbohydrate Digestibility. In *Carbohydrates—Comprehensive Studies on Glycobiology and Glycotechnology*. IntechOpen.

Mitchell, D. A., Rodriguez, J. A., Carrière, F., Baratti, J., & Krieger, N. (2008). An analytical method for determining relative specificities for sequential reactions catalyzed by the same enzyme: Application to the hydrolysis of triacylglycerols by lipases. *Journal of Biotechnology*, *133*(3), 343–350. https://doi.org/10.1016/j.jbiotec.2007.10.012

Monro, J. A., Mishra, S. S., & Hardacre, A. (2011). Glycaemic impact regulation based on progressive geometric changes in solid starch-based food particles during digestion. *Food Digestion*, *2*(1), 1–12. https://doi.org/10.1007/s13228-011-0009-2

Moreau, H., Gargouri, Y., Lecat, D., Junien, J.-L., & Verger, R. (1988). Purification, characterization and kinetic properties of the rabbit gastric lipase. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism*, *960*(3), 286–293. https://doi.org/10.1016/0005-2760(88)90036-7

Mulet-Cabero, A.-I., Egger, L., Portmann, R., Ménard, O., Marze, S., Minekus, M., Le Feunteun, S., Sarkar, A., Grundy, M. M.-L., Carrière, F., Golding, M., Dupont, D., Recio, I., Brodkorb, A., & Mackie, A. (2020). A standardised semi-dynamic in vitro digestion method suitable for food – an international consensus. *Food & Function*, *11*(2), 1702–1720. https://doi.org/10.1039/C9FO01293A

Mulet-Cabero, A.-I., Mackie, A. R., Wilde, P. J., Fenelon, M. A., & Brodkorb, A. (2019). Structural mechanism and kinetics of in vitro gastric digestion are affected by process-induced changes in bovine milk. *Food Hydrocolloids*, *86*, 172–183. https://doi.org/10.1016/j.foodhyd.2018.03.035

Mun, S., Decker, E. A., & McClements, D. J. (2007). Influence of emulsifier type on in vitro digestibility of lipid droplets by pancreatic lipase. *Food Research International*, *40*, 770–781.

Nguyen, G. T., & Sopade, P. A. (2018). Modeling starch digestograms: Computational characteristics of kinetic models for in vitro starch digestion in food research. *Comprehensive Reviews in Food Science and Food Safety*, *17*(5), 1422–1445. https://doi.org/10.1111/1541-4337.12384

Pallares Pallares, A., Loosveldt, B., Karimi, S. N., Hendrickx, M., & Grauwet, T. (2019). Effect of process-induced common bean hardness on structural properties of in vivo generated boluses and consequences for in vitro starch digestion kinetics. *British Journal of Nutrition*, *122*(4), 388–399. https://doi.org/10.1017/S0007114519001624

Pallares Pallares, A., Miranda, B. A., Truong, N. Q. A., Kyomugasho, C., Chigwedere, C. M., Hendrickx, M., & Grauwet, T. (2018). Process-induced cell wall permeability modulates the in vitro starch digestion kinetics of common bean cotyledon cells. *Food & Function*, *9*(12), 6544–6554. https://doi.org/10.1039/C8FO01619D

Parada, J., & Santos, J. L. (2016). Interactions between starch, lipids, and proteins in foods: Microstructure control for glycemic response modulation. *Critical Reviews in Food Science and Nutrition*, *56*(14), 2362–2369. https://doi.org/10.1080/10408398.2013.840260

Pletschke, B. I., Naudé, R. J., & Oelofsen, W. (1995). Ostrich pepsins I and II: A kinetic and thermodynamic investigation. *The International Journal of Biochemistry & Cell Biology*, *27*(12), 1293–1302. https://doi.org/10.1016/1357-2725(95)00092-4

Pluschke, A. M., Jochems, P. G. M., Williams, B. A., Gidley, M. J., Pluschke, A. M., Jochems, P. G. M., Williams, B. A., & Gidley, M. J. (2017). Protection of α-amylase from proteolysis by adsorption to feed components in vitro and in the porcine small intestine. *Animal Production Science*, *58*(4), 640–645. https://doi.org/10.1071/AN15765

Rovalino-Córdova, A. M., Aguirre Montesdeoca, V., & Capuano, E. (2021). A mechanistic model to study the effect of the cell wall on starch digestion in intact cotyledon cells. *Carbohydrate Polymers*, *253*, 117351. https://doi.org/10.1016/j.carbpol.2020.117351

Ruan, Ch.-Q., Chi, Y.-J., & Zhang, R.-D. (2010). Kinetics of hydrolysis of egg white protein by pepsin. *Czech Journal of Food Sciences*, *28*(No. 5), 355–363. https://doi.org/10.17221/228/2009-CJFS

Rutherfurd, S. M. (2010). Methodology for determining degree of hydrolysis of proteins in hydrolysates: A review. *Journal of AOAC INTERNATIONAL*, *93*(5), 1515–1522. https://doi.org/10.1093/jaoac/93.5.1515

Salvia-Trujillo, L., Verkempinck, S. H. E., Sun, L., Van Loey, A. M., Grauwet, T., & Hendrickx, M. E. (2017). Lipid digestion, micelle formation and carotenoid bioaccessibility kinetics: Influence of emulsion droplet size. *Food Chemistry*, *229*, 653–662. https://doi.org/10.1016/j.foodchem.2017.02.146

Sams, L., Paume, J., Giallo, J., & Carrière, F. (2016). Relevant pH and lipase for in vitro models of gastric digestion. *Food & Function*, *7*(1), 30–45. https://doi.org/10.1039/c5fo00930h

Sarkar, A., Murray, B., Holmes, M., Ettelaie, R., Abdalla, A., & Yang, X. (2016). In vitro digestion of Pickering emulsions stabilized by soft whey protein microgel particles: Influence of

thermal treatment. *Soft Matter*, *12*(15), 3558–3569. https://doi.org/10.1039/C5SM02998H

Shin, H., Seo, D.-H., Seo, J., Lamothe, L. M., Yoo, S.-H., & Lee, B.-H. (2019). Optimization of in vitro carbohydrate digestion by mammalian mucosal α-glucosidases and its applications to hydrolyze the various sources of starches. *Food Hydrocolloids*, *87*, 470–476. https://doi.org/10.1016/j.foodhyd.2018.08.033

Sicard, J., Mirade, P.-S., Portanguen, S., Clerjon, S., & Kondjoyan, A. (2018). Simulation of the gastric digestion of proteins of meat bolus using a reaction–diffusion model. *Food & Function*, *9*(12), 6455–6469. https://doi.org/10.1039/C8FO01120F

Tamura, M., Okazaki, Y., Kumagai, C., & Ogawa, Y. (2017). The importance of an oral digestion step in evaluating simulated in vitro digestibility of starch from cooked rice grain. *Food Research International*, *94*, 6–12. https://doi.org/10.1016/j.foodres.2017.01.019

Tester, R. F., Karkalas, J., & Qi, X. (2004). Starch structure and digestibility enzyme-substrate relationship. *World's Poultry Science Journal*, *60*(2), 186–195. https://doi.org/10.1079/WPS200312

Thévenot, J., Cauty, C., Legland, D., Dupont, D., & Floury, J. (2017). Pepsin diffusion in dairy gels depends on casein concentration and microstructure. *Food Chemistry*, *223*, 54–61. https://doi.org/10.1016/j.foodchem.2016.12.014

Tonda, A., Grosvenor, A., Clerens, S., & Le Feunteun, S. (2017). In silico modeling of protein hydrolysis by endoproteases: A case study on pepsin digestion of bovine lactoferrin. *Food & Function*, *8*(12), 4404–4413. https://doi.org/10.1039/C7FO00830A

van Boekel, M. A. J. S. (2008). Multiresponse kinetic modeling of chemical reactions. In *Kinetic Modeling of Reactions In Foods*. CRC Press.

van der Sman, R. G. M., Houlder, S., Cornet, S., & Janssen, A. (2020). Physical chemistry of gastric digestion of proteins gels. *Current Research in Food Science*, *2*, 45–60. https://doi.org/10.1016/j.crfs.2019.11.003

Van Wey, A. S., Cookson, A. L., Roy, N. C., McNabb, W. C., Soboleva, T. K., Wieliczko, R. J., & Shorten, P. R. (2014). A mathematical model of the effect of pH and food matrix composition on fluid transport into foods: An application in gastric digestion and cheese brining. *Food Research International*, *57*, 34–43. https://doi.org/10.1016/j.foodres.2014.01.002

Verger, R., Mieras, M. C. E., & De Haas, G. H. (1973). Action of phospholipase A at interfaces. *Journal of Biological Chemistry*, *248*(11), 4023–4034.

Verkempinck, S. H. E., Salvia-Trujillo, L., Infantes Garcia, M. R., Hendrickx, M. E., & Grauwet, T. (2019). From single to multiresponse modelling of food digestion kinetics: The case of lipid digestion. *Journal of Food Engineering*, *260*, 40–49. https://doi.org/10.1016/j.jfoodeng.2019.04.018

Verkempinck, S. H. E., Salvia-Trujillo, L., Moens, L. G., Carrillo, C., Van Loey, A. M., Hendrickx, M. E., & Grauwet, T. (2018). Kinetic approach to study the relation between in vitro lipid digestion and carotenoid bioaccessibility in emulsions with different oil unsaturation degree. *Journal of Functional Foods*, *41*, 135–147. https://doi.org/10.1016/j.jff.2017.12.030

Verkempinck, S. H. E., Salvia-Trujillo, L., Moens, L. G., Charleer, L., Van Loey, A. M., Hendrickx, M. E., & Grauwet, T. (2018). Emulsion stability during gastrointestinal conditions effects lipid digestion kinetics. *Food Chemistry*, *246*, 179–191. https://doi.org/10.1016/j.foodchem.2017.11.001

Vorob'ev, M. M. (2019). Proteolysis of β-lactoglobulin by Trypsin: Simulation by Two-Step Model and Experimental Verification by Intrinsic Tryptophan Fluorescence. *Symmetry*, *11*(2), 153. https://doi.org/10.3390/sym11020153

Wilkinson, D. J. (2009). Stochastic modelling for quantitative description of heterogeneous biological systems. *Nature Reviews Genetics*, *10*(2), 122–133. https://doi.org/10.1038/nrg2509

Woolnough, J. W., Bird, A. R., Monro, J. A., & Brennan, C. S. (2010). The effect of a brief salivary α-amylase exposure during chewing on subsequent in vitro starch digestion curve profiles. *International Journal of Molecular Sciences*, *11*(8), 2780–2790. https://doi.org/10.3390/ijms11082780

Mathematical modelling of food hydrolysis during *in vitro* digestion: from single nutrient to complex foods in static and dynamic conditions

Steven Le Feunteun, Sarah Verkempinck, Juliane Floury, Anja Janssen, Alain Kondjoyan, Sebastien Marze, Pierre-Sylvain Mirade, Anton Pluschke, Jason Sicard, George van Aken, Tara Grauwet

General considerations

This supplementary material is intended to describe how the enzymatic activities of digestive enzymes were modelled in Figure 2A and 2B of our review paper. Before describing these models, it is noteworthy that the main objective of these figures was to illustrate that the enzymatic activities of digestive enzymes can, at least in principles, be estimated in the changing pH conditions of (semi-)dynamic *in vitro* gastric digestions. Among the three models we used, two have been built for the purpose of the present review: the one for human salivary α-amylase, and the one for rabbit gastric lipase. Both were built on a purely empirical basis (*i.e.* black box). This means that related mathematical equations are not based on mechanistic assumptions, and this is the reason why we do not provide any interpretation for corresponding model parameters.

Estimation of model parameters

The values of model parameters were estimated by fitting model simulations to the corresponding experimental data. All fittings were performed by minimizing the sum of the squared distance between model predictions and experimental data using the solver of Excel 2016 with the Generalized Reduced Gradient method.

Mathematical models of the pH-dependence of gastric enzyme activities (Figure 2A)

Figure 2A illustrates model simulations of the pH-dependence of human salivary α-amylase, porcine pepsin, and rabbit gastric lipase (on long-chain triglycerides), adjusted to the experimental data of (Freitas et al., 2018), (Kondjoyan et al., 2015; Pletschke et al., 1995), and (Moreau et al., 1988), respectively.

Human salivary α-amylase:

The activity of human salivary α-amylase was modelled empirically using an exponential relation as a function of pH:

$$A_{HSA} = 100 \times \ max\{\ 1 - \ exp(-a \ \times (pH - b)), 0\} \qquad (1)$$

where A_{HSA} is the human salivary α-amylase activity (% of the optimum), and where a and b are constants. Model parameters were estimated to be $a = 1.156$ and $b = 3.164$ from the fitting.

Porcine pepsin:

The pH-dependence of porcine pepsin was modelled mechanistically using the model proposed by Kondjoyan, Daudin, and Santé-Lhoutellier (2015). This model relies on the assumption that pepsin behave as a diacid (H_2E), and that its activity is governed by the pK_a of the two aspartic groups composing its reactive site. This model, which further assumes that HE^- is the active form of pepsin, leads to:

$$\frac{HE^-}{E_T} = \frac{1}{1+\frac{10^{-pH}}{K_{a1}}+\frac{K_{a2}}{10^{-pH}}} \qquad (2)$$

with

$$E_T = H_2E + HE^- + E^{2-} \qquad (3)$$

where E_T is the total enzyme concentration, and K_{a1} and K_{a2} are the acidity constants of the two aspartic groups composing the reactive site of pepsin. Model parameters were previously estimated to be $K_{a1} = 2.5^{+1.5}_{-0.9} * 10^{-2}$ and $K_{a2} = 3.2^{+3.1}_{-1.6} * 10^{-3}$ (equivalent pK_{a1} = 1.6 ± 0.2 and pK_{a2} = 2.5 ± 0.3) by Kondjoyan, Daudin, and Santé-Lhoutellier (2015). The pH-dependence of porcine pepsin was therefore modelled as:

$$A_{PP} = 100 \times \frac{\frac{HE^-}{E_T}}{\left(\frac{HE^-}{E_T}\right)_{pH=2.05}} \qquad (4)$$

with A_{PP} is the porcine pepsin activity (% of the optimum, at pH = 2.05 according to the estimated value of K_{a1} and K_{a2}).

Rabbit gastric lipase:

The activity of rabbit gastric lipase was modelled empirically, assuming that it follows a log-normal distribution as a function of pH:

$$A_{RGL} = a \times \text{Lognormal}(\mu, \sigma^2) \qquad (5)$$

where A_{RGL} is the rabbit gastric lipase activity (% of the optimum), a is a scaling factor, μ and σ are the mean and standard deviation of the normal distribution of $\ln(pH)$. Model parameters were estimated to be a = 260.8, μ = 1.457 and σ = 0.256 from the fitting.

In practice, this was performed by applying the LOGNORM.DIST function of Excel on the pH variable.

Mathematical models of the time-dependence of gastric enzyme activities

Figure 2B illustrates the same model simulations but as a function of the gastric digestion time instead of pH. For this, one need to know how the pH evolve as a function of time. In our example, we assumed that the decrease in gastric pH as a function of time follows the model proposed by Van Wey et al. (2014) of the human gastric pH data of Malagelada et al. (1979):

$$pH = 4.179 \times exp(-0.0179 \times t) + 1.4613 \qquad (6)$$

where *t* is the time (min).

In practice, this was performed by substituting the pH variable in Eq. (1), (2) and (5) by Eq. (6).

References

Freitas, D., Le Feunteun, S., Panouillé, M., & Souchon, I. (2018). The important role of salivary α-amylase in the gastric digestion of wheat bread starch. *Food & Function*, *9*(1), 200–208. https://doi.org/10.1039/C7FO01484H

Kondjoyan, A., Daudin, J.-D., & Santé-Lhoutellier, V. (2015). Modelling of pepsin digestibility of myofibrillar proteins and of variations due to heating. *Food Chemistry*, *172*, 265–271. https://doi.org/ham

Malagelada, J.-R., Go, V. L. W., & Summerskill, W. H. J. (1979). Different gastric, pancreatic, and biliary responses to solid-liquid or homogenized meals. *Digestive Diseases and Sciences*, *24*, 101–110. https://doi.org/10.1007/BF01324736

Moreau, H., Gargouri, Y., Lecat, D., Junien, J.-L., & Verger, R. (1988). Purification, characterization and kinetic properties of the rabbit gastric lipase. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism*, *960*(3), 286–293. https://doi.org/10.1016/0005-2760(88)90036-7

Pletschke, B. I., Naudé, R. J., & Oelofsen, W. (1995). Ostrich pepsins I and II: A kinetic and thermodynamic investigation. *The International Journal of Biochemistry & Cell Biology*, *27*(12), 1293–1302. https://doi.org/10.1016/1357-2725(95)00092-4

Van Wey, A. S., Cookson, A. L., Roy, N. C., McNabb, W. C., Soboleva, T. K., Wieliczko, R. J., & Shorten, P. R. (2014). A mathematical model of the effect of pH and food matrix composition on fluid transport into foods: An application in gastric digestion and cheese brining. *Food Research International*, *57*, 34–43. https://doi.org/10.1016/j.foodres.2014.01.002