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Modelling the *in vitro* FooD Digestion SIMulator FooDSIM

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ABSTRACT

Understanding the complexity of human digestion is critical for designing models that serve as valuable research tools for process simulation and prediction. Due to the high cost of medical intervention & recent advancements in *in vitro* digestion protocols, increased demand for inexpensive *in silico* solutions emerges. This study aims to develop a mathematical model that simulates the *in vitro* dynamic Food Digestion SIMulator (FooDSIM) functionalities via a digital twin approach. Ordinary Differential Equations (ODEs) simulate the system as a series of Continuously Stirred Tank Reactors (CSTRs) and describe different regions of human organs (stomach, duodenum, ileum, colon) of the human Gastrointestinal Tract (GIT). Various time horizons were used to investigate the effect of periodic feeding on the dynamic stabilisation of the inherently simulated processes (hydraulics, pH, biochemical interactions between enzymes & substrates, and nutrient absorption). A Polynomial Chaos Expansion (PCE)-based Global Sensitivity Analysis (GSA) was performed to explore causal relationships between input & output variables by performing uncertainty quantification. Results indicate that the system self-stabilizes only after 3-4 feeding cycles. Initially, enzyme concentration increases in the duodenum above the input feed threshold, thus significantly affecting the digestion kinetics in the subsequent reactors. Two cardinal models used for the fitting of enzymatic activity data with pH values indicate satisfactory Mean Squared Error (MSE) scores (<0.0114 for Model 1, <0.0134 for Model 2). Overall, this research highlights the potential of mathematical modeling for simulating digestion and predicting experiment outcomes.

Keywords: Digestion Modeling, Digital Twin, Parameter Estimation, Global Sensitivity Analysis

INTRODUCTION

Human digestion is a complex process that takes place in multiple sites along the human GIT, during which a wide range of digestive fluids and enzymes is secreted. These participate in mechanical and biochemical processes while altering the food matrices to achieve specific goals (e.g. optimal nutrient delivery, local release, and absorption) [1].

However, exploring human digestion is challenging due to the system's complexity. Multiple environmental conditions greatly influence the outcome of human digestion. Inherent variability in samples obtained through *in* vivo studies and limited intervention techniques pose challenging hurdles for exploring human digestion. Therefore, a rise has been given to the development of dynamic *in vitro* digestion systems that offer a simple, fast, and flexible solution to digestion studies.

Besides their advantages, these dynamic *in vitro* systems require laborious and time-consuming experiments. As a response, *in silico* computational approaches are developed to simulate human digestion numerically. That way, predictive information for the *in vitro* systems' inherent digestion processes can be provided. Often, these include highlights not evident during *in vitro* experiments and enable the strategic exploration of research hypotheses [2].

This paper aims to develop a mathematical model for the FooDSIM dynamic *in vitro* simulator by implementing a digital twin approach. The FooDSIM is a laboratory system that consists of four CSTRs connected in series that mimic human digestion. To achieve this, it introduces dynamic processes and reagents to simulate the physiological conditions of digestion. The *in silico* model will simulate the dynamics of these processes by employing a series of ODEs. While both physicochemical and microbial processes aim to be simulated, this paper will primarily focus on describing only physicochemical phenomena.

MATERIALS AND METHODS

Dynamic Food Digestion SIMulator – FooDSIM

General

The exact protocol of development and operation of the FooDSIM laboratory analogue has been previously described by Gaspari et al. (2024) [3]. Thus, within the context of this paper, only some relevant operation features and mathematical formulations that concern the digital twin will be addressed.

The FooDSIM is a computer-controlled, multi-compartmental dynamic *in vitro* simulator that describes major physiological processes within the human GIT. It consists of four Applikon MiniBio bioreactors connected in series that operate as fed-batch or CSTR while representing the stomach, upper/lower small intestine, and colon (Figure 1). Simulated digestive fluids and enzyme solutions are introduced to the respective vessels to simulate the digestion environment. Controlled conditions (37 ^{o}C, pH, oxygen saturation) and continuous stirring led to homogenous mixing. The feeding process takes place through introducing a Food Model System (FMS), which is fed to the 1st vessel. The FMS dynamically interacts with the digestion environment.

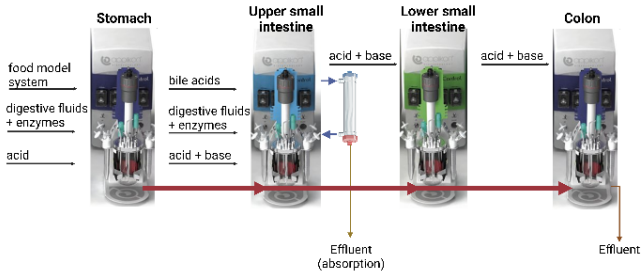

Figure 1. Schematic representation of FooDSIM.

Hydraulics

Emptying Rate

The emptying rate of the 1st vessel (stomach) towards the 2nd (duodenum) is described by the Elashoff emptying rate; an exponential equation that describes the stomach emptying process as a function of time.

$$f(t) = 1 - 2^{-\left(\frac{t}{t_{\frac{1}{2}}}\right)^{\beta}}, \quad (1)$$

where f denotes the fraction of content delivered, t signifies the time (h), and $t_{1/2}$ is the half-time of content emptying, equal to 1,16 hours. Moreover, β is a shape factor equal to 2 and is retrieved from literature [3].

Mass Balance

Every vessel has unique feeding and emptying flow rates. A set of ODEs describes the total mass present in each vessel as a function of time. More specifically:

$$\frac{dM(t)}{dt} = M_{in}(t) - M_{out}(t), \quad (2)$$

where $M\,(g)$ is the mass in every reactor and $M_{in}, M_{out}\,(g/h)$ are the compartment feeding and emptying rates, respectively.

pH Values

The pH in each compartment is actively controlled during every subsequent feeding cycle. More specifically, the pH profile of the stomach follows an exponential decrease from its initial value upon the first introduction of FMS. After the end of each feeding cycle, the controller maintains the low pH of the fasted stomach (pH_{inf}), until the next feeding occurs. There, the pH of the stomach vessel is increasing only due to the buffering effect of the FMS. Once the maximum pH of the fed stomach (pH_o) is reached, the controller is activated again to maintain this pH, and acidification occurs during the stomach's emptying process. The pH in the duodenum, ileum, and colon is fixed and constant. In the stomach, Equation 3 describes the acidification process:

$$pH(t) = pH_o + (pH_{inf} - pH_o) * e^{-\lambda * t}, \quad (3)$$

where λ is an exponential rate equal to 1.5. Every reset of pH between the acidified and initial state is simulated with a linear increase between the upper and lower pH values and lasts for the same time interval as the feeding process.

Digestion Kinetics

Food Model System - FMS

The FMS used in FooDSIM is an oil-in-water emulsion that consists of carbohydrates, fats, proteins, and salts. Its composition, along with the respective molecular weights, is shown in Table 1. The protocol for its formulation is described by Gaspari et al. (2024) [3].

Table 1: Properties of FMS constituents.

Substrate	Concentration (g/L)	Molecular Weight (g/mol)
Soluble Potato Starch	99,00	162,14
Sucrose	37,70	342,30
Dietary Fibre	16,00	300.000,00
Corn Oil	43,00	850,00
Whey Protein	44,40	19.328,00
NaCl	2,50	58,44

Digestion Kinetics

Three enzyme solutions are used to facilitate the enzymatic reactions in FooDSIM, and their main properties are listed in Table 2.

Table 2: Properties of enzymes.

Enzyme	EC Number	Solution Concentration (g/L)	Activity (U/mg)
A-amylase	3.2.1.1	15	50
Pepsin	3.4.23.1	100	400
Pancreatic A-amylase	3.2.1.1	3.75	100
Pancreatic TAG lipase	3.1.1.3	3.75	100
Pancreatic trypsin	3.4.21.4	3.75	100

To simulate the kinetics between available substrates and enzymes in the system, a map of enzyme/substrate specificity is constructed (Table 3).

Table 3: Map of enzyme/substrate specificity. The symbol x indicates affinity between the selected enzyme and substrate.

Substrate	Enzymes		
	A-amylase	Pepsin	Pancreatin
Soluble Potato Starch	x	-	x
Sucrose	-	-	-
Dietary Fibre	-	-	-
Corn Oil	-	-	x
Whey Protein	-	x	x
NaCl	-	-	-

Assuming the system is well stirred, all substrates are instantaneously available to all enzymes in each vessel. Thus, the Michaelis Menten kinetics (4) is used to describe interactions between single enzyme/substrate combinations:

$$r(t) = \frac{Vmax * C_s(t)}{Km + C_s(t)}, \quad (4)$$

where, C_s (g/L) is the dynamic substrate concentration, Km (g/L) is the Michaelis-Menten affinity constant, and $Vmax$ $(g/L*h)$ is the maximum reaction velocity for each combination of enzyme and substrate. When different enzymes degrade simultaneously the same substrate, added terms express each one's kinetics.

The parameter V_{max} is defined as the product of enzyme concentration with respective catalytic reaction rate, as shown in equation 5. This relationship directly assumes that all available enzymes in the reactor are characterized by a maximum catalytic reaction rate when saturated with substrate. Thus, the maximum reaction velocity of the enzyme/substrate reaction kinetics can be calculated. The catalytic reaction rate values for each combination of enzyme/substrate are quantified by utilizing the manufacturer's specifications for enzymatic activity and molecular weight of the substrate, as indicated below:

$$V_{max}(t, pH) = k_{cat} * C_{enz}(t) * AI(pH) \quad (5)$$

$$k_{cat} = U_{enz} * c * MW \quad (6)$$

where, k_{cat} $(1/h)$ is the overall catalytic conversion rate of each enzyme, C_{enz} (g/L) is the enzyme concentration in each compartment, AI $(-)$ is the Activity Index for each enzyme as described further below, U_{enz} (U/mg) is the activity of each enzyme and c $(1/h)$ is a unit conversion factor equal to $6*10^{-5}$.

Enzyme Concentration Balances

Similarly to mass balances, a set of ODEs describes the change of enzyme concentration in every vessel. During the dialysis process, the cut-off limit of the membrane (<5kDa) does not permit the absorption of enzymes. Thus, equation 7 describes the change of enzyme concentration in all reactors except for the 2nd, where dialysis occurs. For that one, a modified ODE (8) accounts for the dynamic accumulation of enzymes, as presented below:

$$\frac{dC_{enz}(t)}{dt} = \frac{M_{in}(t)}{M(t)} * \left(C_{enz,in}(t) - C_{enz}(t)\right) \quad (7)$$

$$\frac{dC_{enz}(t)}{dt} = \frac{M_{in}(t) * \left(C_{enz,in}(t) - C_{enz}(t)\right) + M_{filter}(t) * C_{enz}(t)}{M(t)} \quad (8)$$

where $C_{enz,in}$ (g/L) is the input enzyme concentration in the stomach, and M_{filter} (g) is a term that expresses the mass removed from the compartment due to dialysis.

Effect of pH on Enzymatic Activity

To express the impact of pH on enzymatic activity within the dynamic framework of FooDSIM, the introduction of an Activity Index (AI) takes place. It is calculated by using 2 modified cardinal models, as described by Baka et al. (2013) [4]. The AI can take values between 0-1 and is defined according to the following relationship:

$$AI = \begin{cases} pH < pH_{min}, & 0 \\ pH_{min} < pH < pH_{max}, & AI_{opt} * \rho_i(pH) \\ pH > pH_{max}, & 0 \end{cases} \quad (9)$$

where pH_{min}, pH_{max}, pH_{opt} are the lower, upper, and optimal values for measured enzymatic activity, and AI_{opt} $(-)$ is the optimal AI for each enzyme, respectively. The factor ρ_i is expressed by one of two adjusted nonlinear cardinal models, with one having an inflection point.

$$\rho_1(pH) = \frac{(pH - pH_{min}) * (pH - pH_{max})}{(pH - pH_{min}) * (pH - pH_{max}) - (pH - pH_{opt})^2} \quad (10)$$

$$\rho_2(pH) = \frac{(pH - pH_{min})^2 * (pH - pH_{max})}{(pH_{opt} - pH_{min}) * \left((pH_{opt} - pH_{min}) * (pH - pH_{opt}) - (pH_{opt} - pH_{max}) * (pH_{opt} + pH_{min} - 2 * pH)\right)} \quad (11)$$

Parameter estimation for pH_{min}, pH_{max}, pH_{opt} was performed with least squares regression. Values for pH and residual enzymatic activity were retrieved from the literature for every enzyme. To assess the goodness of fit, the Mean Squared Error (MSE) is used, based on the calculation of the Least Squared Error (LSE):

$$\mathrm{LSE} = \min \sum_{i}^{N} \left(y_{sampled} - y_{(\theta)}\right)^2 \quad (12)$$

$$\mathrm{MSE} = \frac{1}{n_{sampled} - n_{\theta}} * \mathrm{LSE} \quad (13)$$

where $y_{sampled}$ are the measured and y_{θ} are the predicted values of ρ, based on the predicted parameters θ.Moreover, n signifies the amount of sampled data and parameters.

Substrate Concentration Balance

The change in concentration of each substrate in every compartment is described using a set of ODEs (14-15). Like equations 7 and 8, equation 14 accounts for substrate concentration in all reactors except for the 2nd one, where equation 15 describes the accumulation of non-absorbable substrates.

$$\frac{dC_s(t)}{dt} = \frac{M_{in}(t)}{M(t)} * \left(C_{s,in}(t) - C_s(t)\right) - r(t) \quad (14)$$

$$\frac{dC_s(t)}{dt} = \frac{M_{in}(t) * \left(C_{s,in}(t) - C_s(t)\right) + M_{filter}(t) * C_s(t)}{M(t)} - r(t) \quad (15)$$

where $C_{s.in}$ (g/L) is the input substrate concentration and C_s (g/L) is the substrate concentration in each reactor.

Digestion Product Concentration Balance

The concentration of digestion products generated by the degradation of substrates due to enzymatic hydrolysis is described by the following ODE:

$$\frac{dC_p(t)}{dt} = \frac{M_{in}(t)}{M(t)} * \left(C_{p,in}(t) - C_p(t)\right) + r(t), \quad (16)$$

where $C_{p,in}$ (g/L) is the input concentration of digestion products and C_p (g/L) is the concentration of digestion products for each substrate in each compartment.

Dialysis Absorption

To explicitly simulate the absorption of substrates and digestion products during the dialysis in the duodenum phase, the following set of ODEs (17-19) is used:

$$\frac{dM_{filt}(t)}{dt} = M_{filt}(t) \quad (17)$$

$$\frac{dC_{s,filt}(t)}{dt} = C_{s,filt.in}(t) - C_{s,filt}(t) \quad (18)$$

$$\frac{dC_{p,filt}(t)}{dt} = C_{p,filt,in}(t) - C_{p,filt}(t) \quad (19)$$

where, $C_{s,filt}$, $C_{s,filt,in}$ (g/L) express the current and input value of substrates' concentrations. $C_{p,filt,}$ and $C_{p,filt,in}$ express the current and input concentrations of digestion products in the dialysate.

Numerical Simulations

Software and Solver

Numerical simulations for a 7-day operation of FooDSIM were performed with MATLAB R2023a software. The system of ODEs (71 equations) was solved with the ode15s function within 135 s.

Uncertainty Quantification

Global Sensitivity Analysis - GSA

GSA was employed to examine two different scenarios by using the open-source UQLab Sensitivity Analysis Module. Various uniform, independent uncertainty distributions were created for the i) composition of FMS (±10% deviation) and ii) enzyme activity (0-max activity). The proposed uncertainty distribution resembles error during the preparation of the FMS and partial/complete enzyme inactivation, respectively.

Both scenarios quantify the outcome of input uncertainty on the response variables (dialysate digestion product concentration) at an individual level (first order) and by incorporating their interactions (higher order). The sum of them is called total Sobol's sensitivity indices.

Polynomial Chaos Expansion - PCE

Due to the high computational cost of the FooDSIM model, the PCE meta-modelling technique is used to approximate the Sobol decomposition. More specifically, PCE estimates the model's response by an infinite sum of orthogonal basis functions, leading to fewer summands in the decomposition for Sobol's indices estimation. A truncated version of this sum of M terms is presented below:

$$f(\theta) = yPCE = \sum_{i=0}^{M} a_i \Phi_i(\theta) \quad (20)$$

$$M + 1 = \frac{(p+d)!}{p!d!} \quad (21)$$

where Φ is a basis function, a is an unknown coefficient, p is the polynomial order, and d is the number of random inputs [5]. The maximum polynomial degree is defined as 6, and the maximum size of samples is 100 in all cases.

RESULTS AND DISCUSSION

The results for a 7-day simulation of the FooDSIM Digital Twin are presented in Figure 2. Recurring dilution in all compartments leads to a cyclic steady state of FooDSIM. Among all the processes simulated, the dialysis in duodenum is highlighted for achieving an 80% reduction in mass per feeding cycle. Enzymes are generally not absorbed during this process, and their concentrations spike. This concentration increase irreversibly influences the reaction kinetics in all subsequent vessels.

However, the dynamic change of pH impacts the enzymatic/substrate reaction kinetics, as indicated in

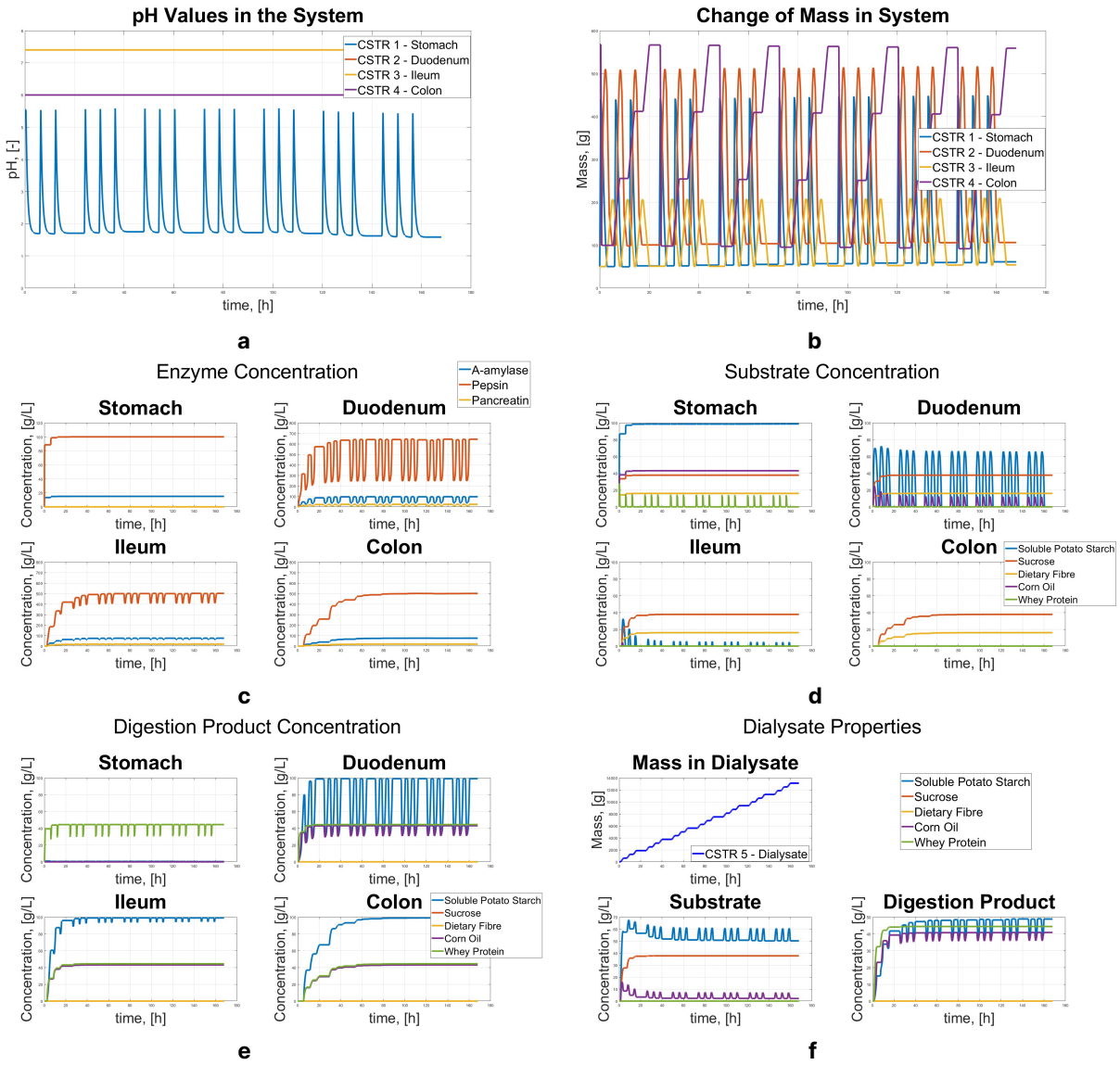

Figure 2: Quantitative results for 7 days of FooDSIM simulation. a) Dynamic pH value in FooDSIM, **b)** Dynamic mass change in FooDSIM, **c)** Dynamic change of three enzyme concentrations, **d)** Dynamic change of five substrate concentrations in FooDSIM, **e)** Dynamic change of digestion products in FooDSIM, **f)** Dynamic change of mass, substrate and digestion products concentration in Dialysate**.**

Figure 3. Parameter estimation using the 2 cardinal models on sampled data exhibits satisfactory goodness of fit. Model 1 describes the activity of α-amylase, pepsin, pancreatic α-amylase, and pancreatic lipase (MSE= 0.0091, 0.0114, 0.0091, 0.0027, respectively). Model 2 is suitable for pancreatic trypsin (MSE = 0.0134) due to the inflection point in its formulation. Therefore, substrates susceptible to enzymatic degradation follow a cyclic profile, too.

Due to the inactivation of salivary α-amylase in the acidified stomach compartment, soluble potato starch is partially consumed and broken down by pancreatic α-amylase instead. It should be noted that all substrates are degraded before the colon. The simulation results indicate that it takes at least 3-4 feeding cycles (1-1,5 days) to achieve a steady regime in the system.

Overall, PCE-based GSA results indicate clear connections within the model's input uncertainties with response variable outcomes, as expected. Total Sobol's indices do not indicate interferences between input variables. The connection between FMS composition and digestion products demonstrates that reaction kinetics occur only between specific enzymes and their allocated substrates. Lastly, GSA for enzyme activity highlights the relative contribution of α-amylase/ pancreatin to degrading soluble potato starch.

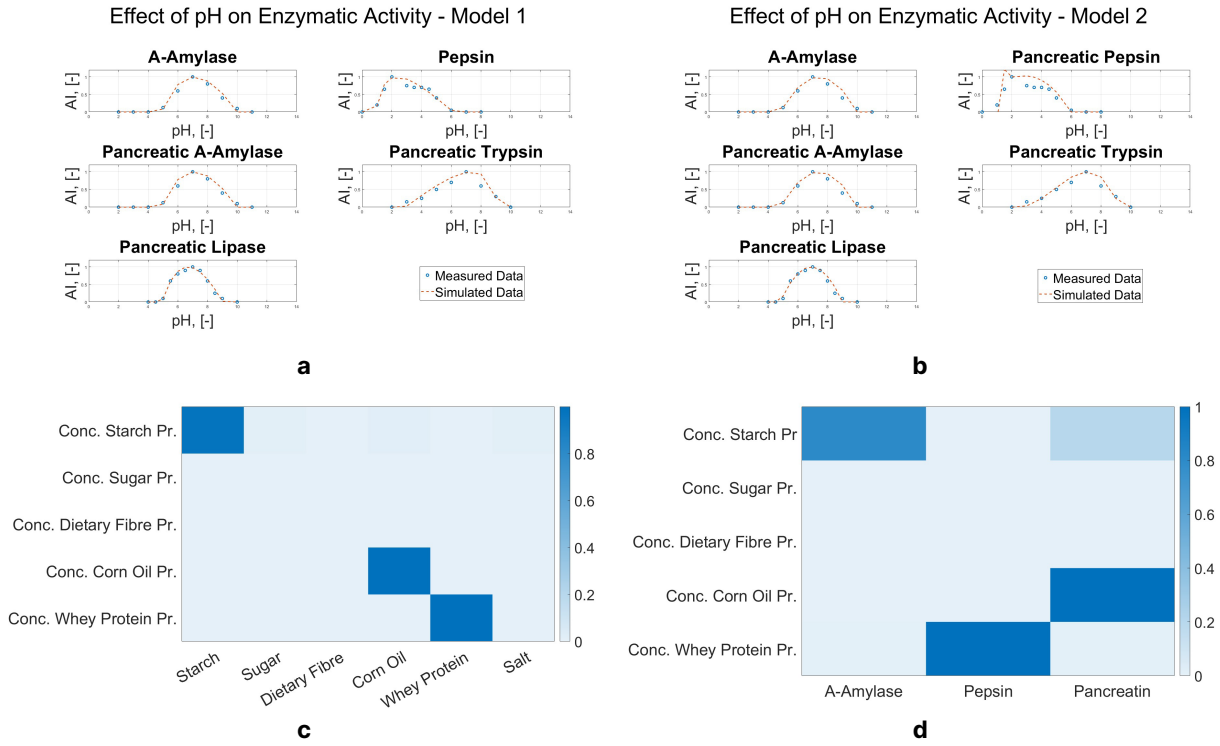

Figure 3: Additional results for parameter estimation and total Sobol indices of PCE-based GSA. a) Effect of pH on enzymatic activity - Cardinal Model 1, **b)** Effect of pH on enzymatic activity - Cardinal Model 2, **c)** Impact of FMS composition on dialysate digestion products concentration, **d)** Impact of enzyme affinity on dialysate digestion product concentration.

CONCLUSIONS

Overall, the FooDSIM Digital Twin provides predictive information that emerges from many parallel physicochemical processes. Its white-box structure permits direct inference and modification of its degrees of freedom. One of the most important outputs is the necessity for at least 3-4 feeding cycles for the system to reach a cyclic steady state regime. This information was hidden and is a critical finding for designing *in vitro* experiments with FooDSIM. Results before the stabilization point could have high inherent variability. The dynamic FooDSIM Digital Twin leads to insights on long-term digestion-related effects. Overall, this model provides a clear view of the physicochemical processes within FooDSIM, and its validation with *in vitro* experiments is a future step.

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