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Studying semi-dynamic digestion kinetics of food: Establishing a computer-controlled multireactor approach

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ABSTRACT

In this work, a multireactor system to study digestion (MuReDi) kinetics is introduced. For this, a custom-made automated system with four independent syringe pumps (BioXplorer 100, H.E.L Group) was acquired. This system consists of multiple, small-scale reactors allowing to study digestion as a function of time and thus to determine digestion kinetics. The different digestion conditions used in the oral, gastric, and small intestinal phase were based on the digestion protocols published by the INFOGEST consortium. We showed that the minimum working volume of a reactor is 30 mL. Besides, repeatability of the digestion kinetics was shown for two food systems: a liquid Ensure® Plus Vanilla drink, and a solid, cooked lentil sample. When comparing static digestion kinetics with semi-dynamic ones, a significantly different digestion pattern was observed. In the static case, a relatively fast hydrolysis rate was observed until a clear plateau was reached. Oppositely, for the semi-dynamic case, a delayed start of the hydrolysis process was noticed. In the gastric phase, this was explained by the decreasing pH and the large pH dependency of pepsin activity. In the small intestine, the lag phase was relatively shorter, yet clearly present. Here we related it to the gradual enzyme (and bile salt) secretion that had to diffuse towards the substrate before hydrolysis could start. Generally, this work showed that the MuReDi system could be used to perform a semi-dynamic digestion approach which largely impacted the overall digestion kinetics. This is important to consider in future *in vitro* food digestion simulation work to come closer to physiologically relevant digestion kinetics.

1. Introduction

One of the key functions of food is to provide compounds to sustain (human) life and health. Food is structured by different compounds, such as macronutrients which need to be released from the food matrix and need to be hydrolyzed through the dynamic process of food digestion to be absorbed (Capuano & Janssen, 2021; Verkempinck, Pallares Pallares, Hendrickx, & Grauwet, 2020). Decades ago, it became obvious that food composition largely influences the metabolites and bioactive compounds made available for absorption and metabolism (Comunian, Drusch, & Brodkorb, 2021). In the last decade, focus of food scientists started to shift from studying food composition towards studying food structural organization and their impact on digestion (kinetics) of, especially, macronutrients (Moughan, 2020).

An important aspect to consider when studying food digestion, is the digestion approach. Throughout the years, different approaches were used to evaluate *in vitro* digestion (Colombo, Ferron, Frosi, & Papetti, 2021). The most employed in this context are static *in vitro* models which are based on the human gastrointestinal physiology, yet being more simple, economical, and of higher throughput than *in vivo* models (Bohn et al., 2017; Colombo et al., 2021). Static *in vitro* digestion models are predominantly used to study food digestion kinetics (i.e.., rate and extent) as influenced by, for example, food composition, structure, or processing. Static models allow to easily interrupt the digestion process to gain mechanistic and kinetic insight. Nevertheless, *in vivo* digestion is a dynamic process. Static models fail to include any time dependency of the digestion conditions within a compartment, which makes static *in vitro* digestion models less suitable for the estimation of more

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physiologically relevant macronutrient digestion kinetics (Bohn et al., 2017; Brodkorb et al., 2019). Important conditions that directly affect digestion kinetics are the dynamic nature of secretions (e.g., digestive enzymes, gastric acid) and the gradual emptying of the stomach. These aspects can also modify, among others, nutrient structural organization, enzymatic activity, and substrate-enzyme contact time (Dupont et al., 2019). Dynamic *in vitro* models consider physical and mechanical processes and time dependent changes occurring in the digestive system. Several dynamic *in vitro* digestion models were developed in the past years, as discussed by several authors (e.g., Sensoy, 2021; Verhoeckx et al., 2015). However, they are often considered to be rather complex, relatively expensive, of low throughput, uninterruptible, and data interpretation is often more challenging. Besides, in many dynamic digestion models, focus is given to mimicking particular dynamic conditions (e.g. mechanical, chemical conditions) rather than mimicking the full complexity of a living being (Sensoy, 2021; Verhoeckx et al., 2015).

In this context, semi-dynamic *in vitro* models came to the attention of researchers as these types of models allow to make strategic choices on which dynamic factors to include into specific phases of the gastrointestinal tract. More specifically, early 2020, a consensus semi-dynamic *in vitro* gastric digestion model for healthy adults was published by the INFOGEST consortium (Mulet-Cabero et al., 2020). This model gives specific attention to mimicking the transient nature of gastric secretions and emptying by use of one single reactor (Mulet-Cabero et al., 2020). However, this model and many other (semi-)dynamic models (e.g., semi-dynamic gastric INFOGEST model, DGM, DIDGI®) (Mulet-Cabero et al., 2020; Verhoeckx et al., 2015), do not allow to take independent samples as a function of digestion time to study digestion kinetics. Collecting independent data is especially important to increase the statistical power of the digestion experiment. Besides, mathematical modelling of such data by means of empirical and/or mechanistic models should also be considered to take next steps towards the construction of predictive *in silico* models.

As a first step in answering the need for digestion studies under more physiologically relevant conditions, we acquired a custom-made computer-controlled system. With this "multireactor system to study digestion kinetics", or MuReDi system, we aimed to establish a semi-dynamic method introducing dynamic secretions of digestive fluids and enzymes during the gastric and small intestinal phase in multiple reactors simultaneously. A series of preliminar tests led to the conditions presented in this work. Dynamic secretions were progressively introduced in the different experiments to study the impact on the digestion kinetics. Repeatability of the MuReDi system was evaluated as well as the impact of working volume on digestion kinetics. For this, two distinct food systems were selected: a liquid Ensure® Plus Vanilla drink, and a solid, cooked lentil sample. Multiple nutrients were evaluated as a function of digestion time from independent reactors to determine and model their digestion kinetics. To the best of our knowledge, such small-scale multireactor system has never been used before for food digestion studies and can be a large step forward in the context of the kinetic evaluation of nutrient digestion under (semi-)dynamic digestion conditions.

2. Materials and methods

2.1. Materials

Ensure® Plus Vanilla drinks were acquired from Sorgente (The Netherlands) and stored at room temperature until use. It is a commercially available drink, rich in a standardized amount of nutrients (4.92% lipids, 20.20% carbohydrates, 6.25% protein per 100 mL according to the label). Dried green lentils (1.86% lipids, 73.80% carbohydrates, 21.65% protein per 100 g dry matter) of the Dupuy-type (L. culinaris), originally grown and harvested in Canada in Augustus 2019, were kindly donated by Casibeans (Melsele, Belgium). The lentils were sorted and cleaned from foreign material, whereafter they were stored at −40 °C to ensure stability until usage (Kyomugasho, Kamau, Aravindakshan, & Hendrickx, 2021). Porcine pepsin (3344 U/mg), pancreas pancreatin (4xUSP) (amylase: 41.0 U/mg, lipase: 36.3 U/mg, trypsin: 3.2 U/mg, chymotrypsin: 1.2 U/mg), trypsin (276.3 U/mg), and chymotrypsin (50.6 U/mg) were bought from Sigma Aldrich (Belgium). All chemicals and reagents used in this work were of HPLC or analytical grade.

2.2. Preparation of cooked lentil sample

Raw lentils were soaked in excess of demineralized water (1:10 w/v) for 16 h at 25 °C. The soaking water was discarded before cooking the seeds in excess of demineralized water (1:10 w/v) for 30 min at 95 °C. This cooking time was selected as it led to a palatable hardness and starch was fully gelatinized (data not shown). The cooking water was discarded before mixing cooked lentils with the electrolyte simulated salivary fluid (eSSF) (2 min, 3000 rpm, IKA® T25 ultra-turrax, Janke and Kunkel, Germany) in a ratio 4:1. The eSSF composition is in accordance to Brodkorb et al. (2019). The mixing represents the mechanical disintegration that would occur during mastication (Pallares, Loosveldt, Karimi, Hendrickx, & Grauwet, 2019). The ratio cooked lentils to eSSF was selected so the sample obtained would represent a bolus (more details in Section 2.3).

2.3. Static in vitro digestion

Prior to performing the digestion studies, the enzymatic activities and bile salt concentration were determined (Section 2.1) (Brodkorb et al., 2019; Minekus et al., 2014). The standardized protocol published by the international INFOGEST consortium was followed to perform static *in vitro* digestions (Brodkorb et al., 2019; Minekus et al., 2014). The only change made was related to the dilution of the oral phase. For this, we followed the guidelines given in the semi-dynamic INFOGEST protocol (Mulet-Cabero et al., 2020) and is based on the dry matter content of the food studied. In more detail, for the Ensure® drink, a 1:1 dilution of food to simulated salivary fluids (SSF; including eSSF, 0.3 M $CaCl_2$, and MilliQ water) was considered. For the cooked lentils, a 1:2 dilution food:SSF was considered to obtain a realistic, paste-like consistency of the bolus (Brodkorb et al., 2019). No amylase was added at this stage as it would be immediately inactivated in the gastric phase due to the static pH 3 (Fried, Abramson, & Meyer, 1987).

The bolus was subsequently mixed with SGF (including electrolyte simulated gastric fluid (eSGF) set at pH 3 and 0.3 M $CaCl_2$) and a pepsin solution of 2000 U/mL of chyme. HCl (2 M) and Milli-Q water were added to reach a pH of 3 and a 1:1 ratio of bolus to simulated gastric fluids (SGF). The small intestinal phase started by adding electrolyte simulated intestinal fluid (eSIF) set at pH 7 and 0.3 M $CaCl_2$. Bile salts were added to reach a 10 mM concentration in the final digest. Pancreatic solution was added to account for 100 U/mL trypsin, 25 U/mL chymotrypsin, 200 U/mL amylase, and 177 U/mL lipase in the final digest. NaOH (1 M) and Milli-Q water were added to reach a pH of 7 and a 1:1 ratio of chyme to simulated intestinal fluids (SIF) (Brodkorb et al., 2019). It must be noted that we opted to not add pure lipase to cover the recommended lipase activity of 2000 U/mL digest when lipid digestion is studied since lipid digestion was only studied in case of the Ensure® drink and we wanted to keep the protocol as similar as possible for the two food types studied within this work. Besides, the lipid content of Ensure® Plus is relatively low (4.92%), so no significant effect on the lipolysis extent was expected based on previous observations (Verkempinck et al., 2022).

The static protocol was performed by means of *(i)* commonly used glass tubes and *(ii)* glass reactors placed into the BioXplorer 100 system. This latter equipment is discussed in more detail in Section 2.4.1. In any case, the digestion was simulated under motion at 37 °C. When using tubes, this was done with a rotating wheel (40 rpm) placed in a heated

incubator (37 °C). For the reactors, the mixing and heat-regulating system of the BioXplorer 100 was used (Section 2.4.1).

Eight individual and thus independent recipients were employed in most cases to be evaluated at eight different time moments (after 5; 10; 20; 30; 45; 60; 90; or 120 min of enzyme addition in the gastric or small intestinal phase). At each indicated time, enzymes were inactivated, allowing to study the time dependent digestion behavior of each macronutrient under evaluation. In the gastric phase, the pH was increased to pH 8 to inhibit pepsin activity (Egger et al., 2016). In the small intestinal phase, a distinction was made between the food containing lipids (Ensure® Plus) and the one with a negligible amount of lipids (lentils). For the Ensure® drink, an aliquot of the digest was inhibited by adding 4-bromophenylboronic (1 M in methanol) to inhibit pancreatic lipase (Grundy et al., 2021). Another aliquot was placed in a water batch (5 min, 98 °C) to inhibit proteases and amylase (Brodkorb et al., 2019). The latter approach was also followed for the lentil samples.

2.4. Semi-dynamic in vitro digestion

In this section, the BioXplorer 100 equipment will firstly be introduced (Section 2.4.1). Thereafter, the conditions used to simulate semi-dynamic digestion in this study, are given (Section 2.4.2). Finally, the translation of these digestion conditions into a plan that could be run with the MuReDi software is explained (Section 2.4.3).

2.4.1. BioXplorer 100 equipment

The BioXplorer 100 equipment (H.E.L Group, London, U.K.), further called 'MuReDi system' since it is a multireactor system employed to simulate digestion kinetics, is a computer-controlled multireactor system with eight independent reactor zones and was custom-tailored (Fig. 1). The system is composed of a panel with a reactor block which

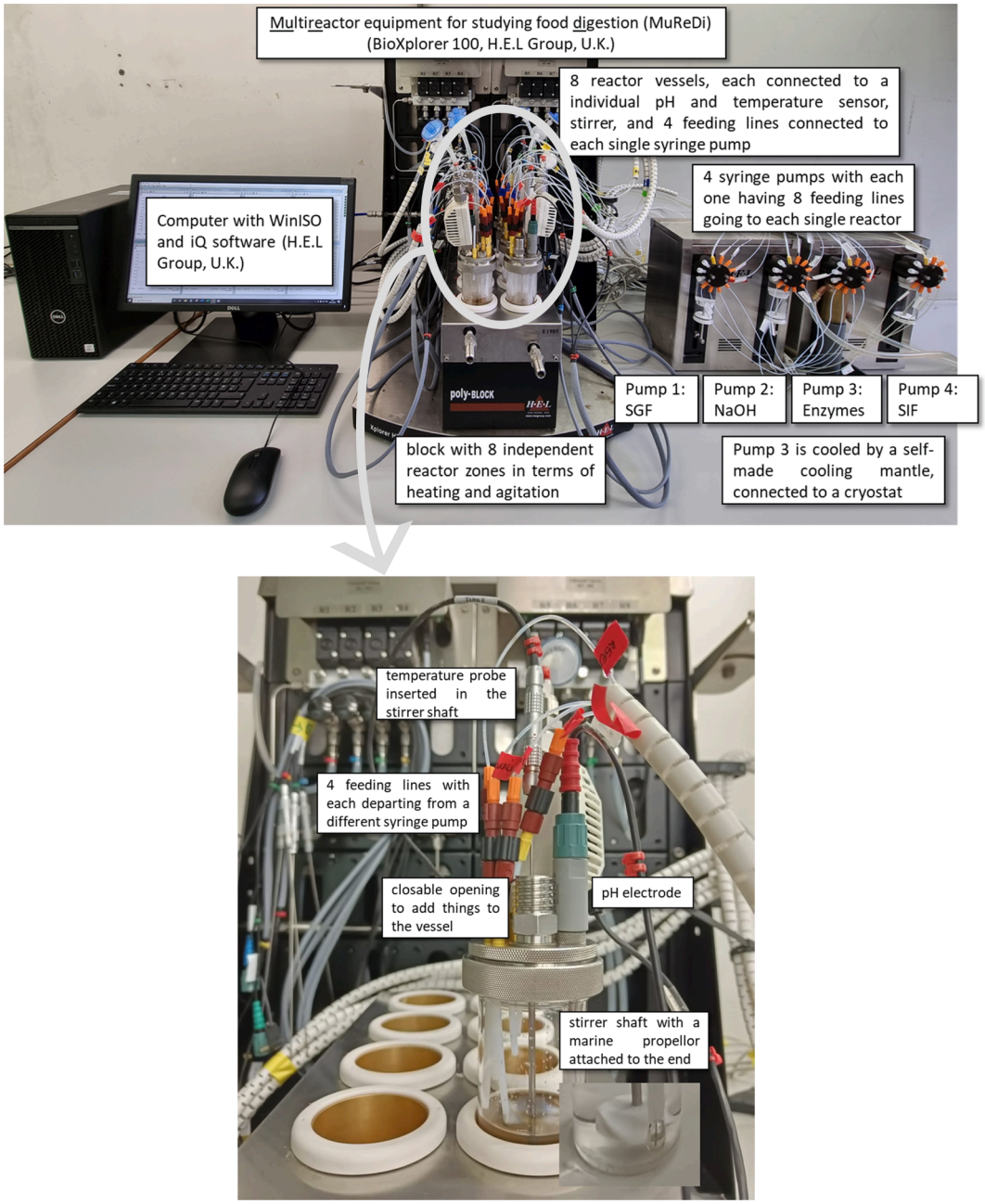

Fig. 1. The multireactor equipment used in this work to introduce semi-dynamic digestion conditions.

has eight independent reactor zones in terms of heating and agitation. The accuracy of temperature measurement is ±0.1 °C according to the manufacturer and is continuously measured inside each reactor by a temperature probe located in the stirrer shaft and in each zone surrounding the reactors. This MuReDi system can handle temperatures ranging from 20 to 200 °C. Magnetic drive agitation is included, and different impellor types can be installed. In this study we used marine impellors. The stirring speed can be varied from 250 to 1500 rpm, but effectively reaching the set speed largely depends on the sample amount and type. Reactor vessels with a total volume of 200 mL were employed. Each reactor is covered with a custom-made lid having several ports connecting to the stirrer, pH probe, temperature sensor, feeding lines etc. (Fig. 1). Indeed, each vessel is equipped with a pH sensor (measuring range of 1–12, temperature range of 0–135 °C) allowing real time monitoring and thus real time feedback. Besides, each vessel is coupled to four liquid feeds. These are in turn coupled to four independent syringe pumps with the ability to add liquid to each reactor according to a pre-set flow rate (mL/min) (0.005–50 mL/min for the 5 mL syringe volume, 0.02–600 mL/min for the 50 mL syringe volume). One of these syringe pumps is surrounded by a self-made cooling mantle coupled to a cryostat allowing to pump a cooled solution into the vessels (e.g., enzyme solutions). For clarity, each syringe pump has eight feeding lines of which one is connected to each reactor lid. In the end, each reactor lid can relate to four feeding lines, each departing from a different syringe pump. Lastly, each lid has a closable opening which can be used to add a liquid to the vessel (Fig. 1).

The software control system (WinISO) allows to write a multistep plan indicating which parameter(s) must be monitored and controlled, including, but not limited to, stirring speed, pH level, pumping rate of liquid feeds, and temperature. Data are continuously logged per reactor and can be processed using the iQ software provided by the manufacturer (e.g., Figure A and B, Supplementary Material).

2.4.2. Semi-dynamic digestion conditions

A series of preliminar experiments were performed to decide which conditions would be used in this study. To be clear, only the final selection of conditions is presented in this work.

As stated in Section 2.3, we only focused on mimicking the dilution of the oral phase by mixing the food with SSF in the ratios mentioned before. At this stage, we also did not include amylase as we wanted to keep the procedure as simple as possible to proof the working principle of the MuReDi system in this digestion context. Besides, excluding amylase at this point allowed a better comparison of static and semi-dynamic digestion kinetics in the discussion part.

The semi-dynamic digestion conditions applied during the gastric phase were based on the recommendations given by the INFOGEST network (Mulet-Cabero et al., 2020). A SGF master mix including eSGF (pH 3), $CaCl_2$, HCl, and MilliQ water was prepared. The ratio HCl to MilliQ water was determined in a preliminar experiment to reach a pH of 2. For the Ensure® drink, the pH of the master mix was 0.94 ± 0.01, while for the lentil samples this was 1.09 ± 0.04. Pumping these acidic SGF solutions allowed a gradual decrease in pH during the gastric phase. A pepsin solution was prepared to provide a final activity of 2000 U/mL. We performed the gastric phase in two ways. First, we evaluated the sole effect of decreasing the pH from the original food pH to pH 2 over 2 h of gastric phase on protein digestion kinetics. Secondly, and in similarity to the INFOGEST protocol, we gradually decreased the pH as well as gradually added pepsin as a function of gastric time. In both cases, after the oral phase, a basal gastric volume of SGF (10%) was added to the bolus. Thereafter, the remaining 90% of SGF was gradually added as function of gastric digestion time. In case also pepsin was gradually added, the pepsin solution was gradually pumped into each reactor by means of the cooled syringe pump. No gastric lipase was added since lipids are mainly digested in the small intestinal phase (>70%), and the only full-fledged alternative to human lipase, rabbit gastric lipase, is still rather expensive given the relatively high working volumes in the MuReDi system. Besides, the lipid content of Ensure® Plus Vanilla was relatively low. In future, it is worth considering the addition of gastric lipase during (semi-dynamic) gastric digestion simulation. In similarity to the static digestion protocol used, pepsin was inactivated by increasing the pH to 8 at the pre-determined time moments.

The small intestinal phase was also simulated in a semi-dynamic way. We made the strategic choice to simulate this phase semi-dynamically since food enters the small intestine in a gradual way and thus is continuously exposed to the addition of enzymes and bile salts. For the approach, we based ourselves on the guidelines and concepts described in the different INFOGEST protocols (Brodkorb et al., 2019; Mulet-Cabero et al., 2020). In more detail, a SIF master mix including eSIF (pH 7), $CaCl_2$, MilliQ water, and bile salts (10 mM in final digest) was prepared. NaOH (2 M) was pumped separately to increase the pH to 7. Besides, a pancreatic solution containing 100 U/mL trypsin, 25 U/mL chymotrypsin, 200 U/mL amylase, and 177 U/mL lipase was prepared and pumped using the cooled syringe pump. At the start of the small intestinal phase, first a basal volume of SIF (10%) was pumped into each reactor. Secondly, the pH was increased to pH 6.6. A slightly lower pH was set at this stage to avoid overshooting by the multireactor system. Finally, the remaining master mix (90%) and pancreatic solution were delivered gradually over 2 h, and the pH was further increased to 7. It must be noted that at the end of the small intestinal phase, the ratio chyme:SIF was not exactly 1:1 since the amount of NaOH (2 M) needed to reach pH 7 was case-dependent and thus could not be accounted for beforehand. However, based on the data obtained, this NaOH volume was rather limited (<2% total volume after 2 h of intestinal phase). Besides, the volume of the reactor content is continuously monitored by the software and thus accounted for when making calculations.

In similarity to the static approach, eight individual and thus independent vessels were employed to be evaluated at eight different time moments (after 5; 10; 20; 30; 45; 60; 90; or 120 min since the start of enzyme addition in the gastric or small intestinal phase). Enzymatic reactions were stopped through chemical inactivation in a particular vessel to allow the evaluation of digestion kinetics (i.e., kinetic approach). Enzyme inactivation was performed as described in Section 2.3.

2.4.3. BioXplorer 100 settings

A multistep plan was written in the WinISO software according to the conditions that we aimed to simulate as described in Section 2.4.2. In total, five plans were written: static gastric phase, static small intestinal phase, gradual pH change in the gastric phase, semi-dynamic gastric phase (i.e., gradual addition of gastric fluids and enzyme), and the combination of a semi-dynamic gastric and small intestinal phase. The plans are represented in a table format in Supplementary Material (Table A). For both static digestions, the plan was only used to control temperature (37 °C), stirring speed (250 rpm), and time (5–120 min). For the semi-dynamic digestions, the plan started with a 9 min lasting equilibration/oral phase step in which the temperature was increased to 35 °C, stirring was started (250 rpm) and visually checked, and SSF was manually added through the closable opening of the lid. It is important to note that no enzymes were added at this stage. The termination step used here was time and temperature, this means that when these 9 min were over and the temperature was at least 35 °C, the plan went to the second step. In the second step, the basal SGF volume was pumped as fast as the system allowed in each reactor to continue to step 3 (<1 min). In the third step, the remaining SGF solution (90%) was pumped linearly over 2 h using one of the feeding lines connected to a syringe pump (P1) (0.2125 mL/min when started from a 30 mL oral phase). Simultaneously, the pepsin solution was pumped at once or gradually spread over 2 h, depending on the experiment, using the feeding line connected to the cooled syringe pump (P3) (0.0125 mL/min when started from a 30 mL oral phase). If a kinetic evaluation in the gastric phase was the goal of the study, time was the termination step used. This means the pumps stopped pumping any liquid into the reactor at pre-set times and

control (not monitoring) of temperature, stirring, and pH stopped. The enzymes were manually inactivated as described earlier (Section 2.3). If a kinetic evaluation in the small intestinal phase was the goal of the study, the termination step of the gastric phase was set at 120 min, whereafter the plan continued to step 4. In the fourth step, a basal volume of SIF was pumped (P4) into each reactor as fast as the system allowed to continue to step 5 (<4 min). In the fifth step, the pH of a reactor was increased to pH 6.6 (P2). The pH was set slightly lower to avoid overshooting by the system. When this pH value was reached, the plan moved to the sixth and final step of the plan. In the sixth step, the remaining SIF solution was pumped linearly over 2 h (0.325 mL/min when started from a 30 mL oral phase). Simultaneously, the pancreatic solution was pumped gradually over 2 h using again the feeding line connected to the cooled syringe pump (P3) (0.125 mL/min when started from a 30 mL oral phase), and the pH was further increased to pH 7 (P2).

Prior to the start of the experiments, all solutions needed during the experiment were prepared and used to prime the feeding lines. After priming, all feeding lines were connected to the lids of each reactor (4 feeding lines, each departing from a different syringe pump). In other words, each syringe pump had a line going to each reactor resulting in maximally four feeding line connections to each reactor (Fig. 1).

2.5. Protein digestion quantification

Digested samples were first centrifuged (10 min, 2000g, Sigma 4–16 KS, Sigma, Osterode am Harz, Germany) to collect the digested fraction in the supernatant. Secondly, the readily bioaccessible fraction ($NH_{2\,(TCA)}$) and, in one selected case, the readily bioaccessible fraction ($NH_{2\,(TCA,hydrolyzed)}$) were determined as described by Pälchen et al. (2021). For this, the *o*-phthaldialdehyde (OPA) spectrophotometric assay was used (Nielsen, Petersen, & Dambmann, 2001; Zahir, Fogliano, & Capuano, 2018). Lastly, the protein hydrolysis was expressed as readily bioaccessible protein (%) (Eq. (1)) and, in one selected case, the readily $\text{bioaccessible}_{\text{hydrolyzed}}$ protein (%) was determined as well (Eq. (2)).

$$Readily\ \ bioaccessible\ \ protein\,(\%) = \frac{NH_{2(TCA)} - NH_{2(initial)}}{NH_{2(total)} - NH_{2(initial)}} \times 100 \quad (1)$$

$$Readily\ \ bioaccessible_{hydrolyzed}\ \ protein\,(\%) = \frac{NH_{2(TCA,hydrolyzed)} - NH_{2(initial)}}{NH_{2(total)} - NH_{2(initial)}} \times 100 \quad (2)$$

2.6. Lipid digestion quantification

First, lipid extraction was carried out for the Ensure® drink and its digest samples as described before by Verkempinck et al. (2018). Secondly, multiple lipid digestion species were quantified with an HPLC system coupled to a Charged Aerosol Detector according to the procedure described by Guevara-Zambrano et al. (2022). The quantified species and their respective high purity standards were oleic acid- and linoleic acid-derived species. These are the most abundant fatty acids present in canola and corn oil, used in the Ensure® Plus Vanilla formulation. Finally, the percentage of lipid hydrolysis (%) was calculated based on all reaction products quantified and represents the ratio of hydrolyzed bonds over the total number of hydrolysable bonds (Eq. (3)).

$$Lipid\ \ hydrolysis\,(\%) = \frac{FFA}{3 \times TAG + 2 \times DAG + MAG + FFA} \times 100 \quad (3)$$

2.7. Starch digestion quantification

Also in this case, the digested samples were first centrifuged (10 min, 2000g, Sigma 4–16 KS, Sigma, Osterode am Harz, Germany) to collect the digested fraction in the supernatant. Secondly, the reducing sugar concentration (expressed as maltose concentration) was measured according to the dinitrosalicylic procedure of Miller (1959) and Pallares et al. (2019). A conversion factor of 0.95 was used to calculate starch equivalents from the maltose concentration. Finally, starch digestion was calculated according to Eq. (4).

$$Digested\ \ starch(\%) = \frac{maltose\ \ concentration \times 0.95}{total\ \ starch\ \ content} \times 100 \quad (4)$$

2.8. Data analysis

The digestion of each food was evaluated as a function of digestion time using independent reactors. This means all evaluations (i.e., eight per time moment per food) belong to the characterization of the same food and thus can be analyzed together. In other words, these evaluations can be considered repetitions from a statistical point of view and contribute to one overall food digestion characterization (Verkempinck et al., 2018). The experimental data of each kinetic digestion study were modelled using nonlinear regression and the software JMP (JMP Pro16, SAS Institute Inc., Cary, NC, USA). It must be noted that the semi-dynamic evaluations in the gastric phase could not be modelled.

Depending on the digestion approach used, static *versus* semi-dynamic, empirical models were used to integrate and compare data and different empirical models presented a best fit, depending on the case. For the static cases, a fractional conversion model was selected (Eq. (5)) in accordance to previous studies performed at our research unit (Pälchen et al., 2021; Verkempinck et al., 2018). For the semi-dynamic small intestinal cases, the modified Gompertz equation was selected (Eq. (6)) (Zwietering, Jongenburger, Rombouts, & van 't Riet, 1990).

$$C = C_f + \left(C_i - C_f\right) \times e^{-kt} \quad (5)$$

$$C = C_f \times e^{\left(e^{\left(\frac{k \times e}{C_f}\right)} \times \left(t_{lag} - t\right) + 1\right) \times (-1)} \quad (6)$$

In Eq. (5), C (%) is the predicted hydrolysis at time t (min), C_i (%) is the initial hydrolysis extent, C_f (%) is the final, plateau value reached under the given conditions, k (min^{-1}) is the reaction rate constant, and t (min) is the time in the simulated gastric or small intestinal phase (Verkempinck et al., 2018). In Eq. (6), C (%) is the percentage of hydrolyzed TAG, protein or starch at a time t (min), k (min^{-1}) is the reaction rate constant, C_f (%) is the final, plateau value reached under the given conditions, t_{lag} (min) is the lag time before hydrolysis starts, and t (min) is the time in the simulated gastric or small intestinal phase (Infantes-Garcia, Verkempinck, Saadi, Hendrickx, & Grauwet, 2022; Zwietering et al., 1990). Confidence intervals (95%) were used to determine significant differences among parameter estimates (Verkempinck et al., 2018).

3. Results and discussion

This part consists of three subsections. In the first subsection, *in vitro* digestion kinetics are compared when evolving from the classic, manual *in vitro* digestion approach towards an automated one using the MuReDi system. Secondly, for an Ensure® drink, several elements were evaluated: *(i)* the minimum working volume and repeatability of the digestion kinetics when using different starting volumes in the oral phase, *(ii)* the impact of sampling from a single reactor on the digestion kinetics and repeatability of such an approach, and *(iii)* semi-dynamic digestion kinetics in both the gastric and small intestinal phase. In the third and final subsection, cooked lentils were used to check the repeatability of *in vitro* digestion kinetics and the semi-dynamic digestion kinetics in both the gastric and small intestinal phase are discussed.

3.1. From a conventional to automated in vitro static digestion approach

A first element we aimed to evaluate was how the way temperature

and mixing was controlled by the MuReDi system impacted static digestion kinetics. For this, we performed a static *in vitro* digestion both using commonly used glass tubes and glass reactors that fit into the multireactor system. One difference between those two digestion approaches was related to the scale at which the digestion procedure was performed. In the tubes, the total digest had a volume of 10 mL, while the total digest had a volume of 80 mL in the reactors. For the tubes, a rotating wheel was used to mix the sample with digestive solutions, that was placed into a heated incubator (37 °C). For the reactors, a marine impellor was attached to the stirrer shaft and placed near the bottom of each reactor. The minimum stirring speed of 250 rpm was used. Temperature was controlled by the heating system of the multireactor block and was monitored inside each reactor. We studied the impact on the macronutrient digestion kinetics within two different food types: Ensure® Plus Vanilla, a liquid nutrition drink, and cooked lentils, a solid plant-based food part of the planetary healthy diet (Willett et al., 2019).

In Fig. 2, both the experimental (symbols) and modelled (lines) data for each nutrient studied are represented as a function of digestion time for both food systems studied, as well as for both ways of *in vitro* static simulations. For the Ensure® drink, around 12% readily bioaccessible protein was released by the end of the gastric phase for both static approaches (Table 1). This amount further increased rapidly in the small intestinal phase until 44–51%. Lipolysis was extremely fast in the small intestine and reached levels above 60% already after 5 min of pancreatic lipase addition. The Ensure® drink is a nutrient-rich drink often given to people that are hospitalized and/or experienced undesired weight loss. In practice, this means this drink contains accessible nutrients which can be digested relatively fast, as reflected in the fast static digestion kinetics (Fig. 2A-B, Table 1 and 2). This fast lipolysis is in line with previous research on protein-containing low-lipid emulsions (Infantes-Garcia et al., 2022).

Similarly for the cooked lentils, around 11% readily bioaccessible protein were released by the end of the gastric phase for both static approaches (Fig. 2C, Table 1). This amount increased in the small intestinal phase until a value of 53–57%. Around 92–96% of all starch was hydrolyzed after 2 h of small intestinal phase (Fig. 2D, Table 3). This is line with previous research on diverse pulse types (Edwards et al., 2020; Gwala, Pallares Pallares, Pälchen, Hendrickx, & Grauwet, 2020; Pälchen et al., 2022).

Only negligible differences in parameter estimates (e.g., C_f of lipid and starch digestion, Table 1) were observed among the two static digestion approaches. This shows that when using an automated approach, like the MuReDi system, had little impact on the overall digestion pattern.

3.2. Ensure® Plus Vanilla

3.2.1. Determination of minimum working volume and repeatability of the digestion kinetics using different reactor volumes

A second element we aimed to evaluate, was the minimum working volume of the multireactor system. Downscaling the digestion volume has the advantage that less (pure) enzymes and food are needed during the experiment which reduces overall costs and chemical waste streams. Firstly, pH control had to be checked since the pH electrode must be submerged into the digested sample to allow proper monitoring. The measuring zone of the pH meter was submerged when the starting

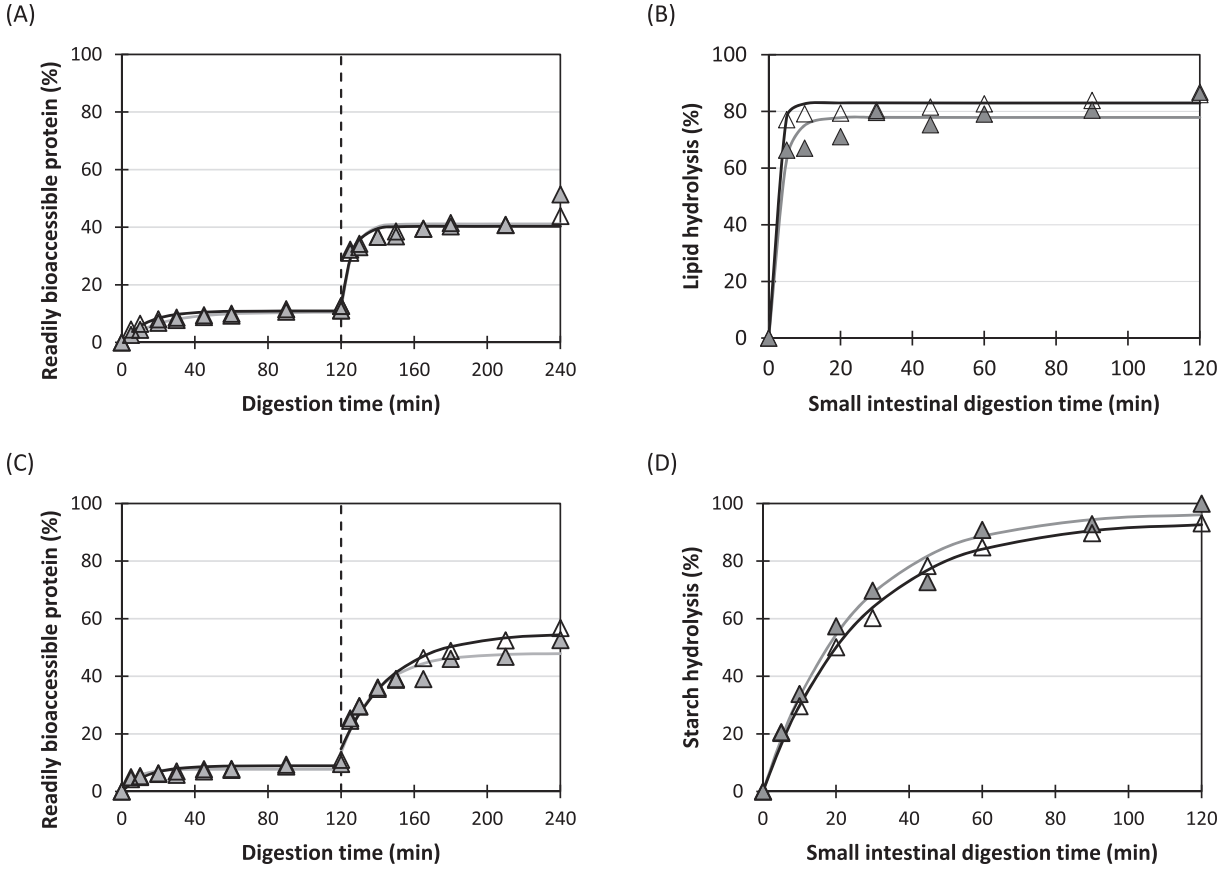

Fig. 2. Time dependent evolution of nutrient hydrolysis (%) quantified as a function of *in vitro* digestion time under static conditions. Symbols represent the experimental values for digestion in ▲ tubes and △ reactors. (A) Release of readily bioaccessible protein and (B) lipid hydrolysis (%) were quantified for Ensure® Plus Vanilla. (C) Release of readily bioaccessible protein (%) and (D) starch hydrolysis were quantified for cooked lentils. Symbols represent experimental data, while lines represent predicted values of the corresponding fractional conversion model (Eq. (5)). The vertical dashed line in (A) and (C) indicates the transition from the gastric to the small intestinal phase.

Table 1
Estimated kinetic parameters for protein digestion in the small intestinal phase of the two food systems digested under different digestion conditions. C_f is the final extent of bioaccessible protein estimated by the model (%), C_i (%) is the initial extent of bioaccessible protein at the start of the small intestinal phase, k is the reaction rate constant of the release of bioaccessible protein (min^{-1}), and t_{lag} represents the potential lag time before bioaccessible proteins are released (min). Different letters indicate significant differences among parameter estimates according to their confidence intervals (95%).

	C_i (%)				C_f (%)				k (min^{-1})				t_{lag} (min)				R^2
ENSURE® PLUS VANILLA																	
Independent tubes/reactors:																	
Static tubes	12.10	±	5.08	A	41.16	±	2.22	A	0.166	±	0.072	A	NA				0.830
Static reactors	13.20	±	2.30	A	40.31	±	1.00	A	0.175	±	0.038	A	NA				0.954
Independent reactors:																	
Repetition 1	NA				47.69	±	2.24	b	0.762	±	0.119	a	17.31	±	3.65	a	0.984
Repetition 2	NA				48.03	±	2.72	b	0.716	±	0.134	a	6.94	±	4.66	b	0.974
Repetition 3	NA				51.75	±	2.42	a	0.831	±	0.126	a	3.89	±	3.46	b	0.979
Sampling from 1 vessel:																	
Average behavior	NA				41.31	±	1.81	a′b′	0.757	±	0.128	a′	12.27	±	3.48	a′	0.983
Repetition A	NA				39.99	±	1.96	b′	0.741	±	0.144	a′	12.80	±	3.93	a′	0.982
Repetition B	NA				41.30	±	1.78	a′b′	0.722	±	0.115	a′	11.37	±	3.41	a′	0.985
Repetition C	NA				42.62	±	1.73	a′	0.809	±	0.129	a′	12.61	±	3.21	a′	0.986
COOKED LENTILS																	
Independent tubes/reactors:																	
Static tubes	13.57	±	3.36	A	47.94	±	2.60	B	0.051	±	0.015	A	NA				0.932
Static reactors	14.81	±	2.20	A	54.94	±	2.34	A	0.036	±	0.007	B	NA				0.977
Independent reactors:																	
Repetition 1	NA				48.70	±	2.38	a	0.659	±	0.084	b	12.25	±	3.41	a	0.987
Repetition 2	NA				66.46	±	4.22	b	0.703	±	0.071	b	12.57	±	3.23	a	0.990
Repetition 3	NA				66.10	±	2.21	b	0.793	±	0.052	a	14.63	±	1.93	a	0.996
Hydrolyzed readily bioaccessible protein:																	
	NA				82.87	±	4.26		0.941	±	0.149		20.04	±	4.17		0.987

Table 2
Estimated kinetic parameters for lipid digestion of the Ensure® Plus Vanilla drink digested under different digestion conditions in the small intestinal phase. C_f is the final extent of lipolysis estimated by the model (%), k is the reaction rate constant of the lipolysis process (min^{-1}), and t_{lag} represents the potential lag time before lipolysis starts (min). Different letters indicate significant differences among parameter estimates according to their confidence intervals (95%).

	C_f (%)				k (min^{-1})				t_{lag} (min)				R^2
Independent tubes/reactors:													
Static tubes	77.87	±	2.21	B	0.332	±	0.077	B	NA				0.401
Static reactors	82.97	±	0.86	A	0.556	±	0.094	A	NA				0.375
Independent reactors:													
SIP 80 mL	79.47	±	2.92	a	2.150	±	0.281	a	21.28	±	2.35	a	0.990
SIP 120 mL	83.24	±	3.27	a	2.232	±	0.337	a	10.55	±	2.67	b	0.983
SIP 160 mL	80.59	±	3.16	a	2.270	±	0.344	a	9.57	±	2.55	b	0.985
Sampling from 1 vessel:													
Average behavior	90.56	±	5.33	a′	2.456	±	0.524	a′	6.04	±	3.68	a′	0.965
Repetition a	90.59	±	5.43	a′	2.280	±	0.465	a′	4.74	±	3.72	a′	0.965
Repetition b	90.16	±	5.33	a′	2.398	±	0.505	a′	5.73	±	3.68	a′	0.966
Repetition c	90.53	±	5.03	a′	2.883	±	0.677	a′	8.57	±	3.58	a′	0.964

Table 3
Estimated kinetic parameters for starch digestion in the small intestinal phase of the cooked lentil sample digested under different digestion conditions. C_f is the final extent of amylolysis estimated by the model (%), k is the reaction rate constant of the amylolysis process (min^{-1}), and t_{lag} represents the potential lag time before amylolysis starts (min). Different letters indicate significant differences among parameter estimates according to their confidence intervals (95%).

	C_f (%)				k (min^{-1})				t_{lag} (min)				R^2
Independent tubes/reactors:													
Static tubes	96.77	±	3.03	A	0.041	±	0.004	A	NA				0.981
Static reactors	93.56	±	1.75	B	0.038	±	0.002	A	NA				0.995
Independent reactors:													
Repetition 1	86.15	±	3.37	b	1.221	±	0.088	a	19.40	±	2.04	a	0.996
Repetition 2	100.60	±	3.54	a	1.295	±	0.069	a	20.76	±	1.59	a	0.998
Repetition 3	100.41	±	3.47	a	1.303	±	0.069	a	20.74	±	1.58	a	0.998

volume (i.e., oral phase) was 20 mL. Stirring speed and temperature control were little impacted by the reactor volume as the magnetic stirrer was always located very close to the reactor bottom (<5 mm) and temperature was monitored by a temperature probe inserted in the stirrer shaft. Besides, we checked if the working volume impacted the digestion kinetics since the working volume directly impacts the rate with which the digestive solutions were pumped to maintain the dilution ratios as suggested in the different INFOGEST protocols (Brodkorb et al., 2019; Mulet-Cabero et al., 2020).

For this, we studied three starting working volumes (oral phase volumes): 20; 30; or 40 mL. This results into a chyme of 40; 60; or 80 mL after 2 h of gastric simulation and subsequently a digest of 80; 120; or 160 mL after 2 h of small intestinal simulation. The data obtained are shown in Fig. 3 and the estimated parameters are given in Table 1 and 2. For both protein and lipid digestion, significant differences were observed in terms of hydrolysis kinetics as impacted by the working volume, especially in the case of the 20 mL oral phase (Table 2). More specifically, significantly longer lag times were noticed for the lowest oral phase volume used which we related to the lower pumping rates used when less volume had to be pumped over a similar time frame (120 min). Based on these insights, the minimum starting volume to perform semi-dynamic digestion kinetics was defined as 30 mL.

3.2.2. *Impact of sampling from a single reactor on digestion kinetics*

Another element we aimed to study, was the possibility to study digestion kinetics by sampling from one reactor instead of using eight independent reactors. Sampling from a single reactor is a commonly used approach in digestion experiments and has the advantage of reducing the overall costs. This, however, results in correlated data demanding for multiple repetitions to increase statistical power.

For this experiment, we increased the working volume so the ratio sampling volume to total volume would be as low as possible, yet enough sample was collected to perform the desired quantifications. In practice, the sampling volume was 3 mL, which was always less than 3.5% of the total (remaining) reactor volume. We performed the digestion experiment in triplicate *(i)* to evaluate the repeatability and *(ii)* to compare digestion kinetics of an experiment in which one reactor was used (correlated data) *versus* an experiment in which eight independent reactors were used (uncorrelated data).

As observed from Fig. 4 and Table 1 and 2, the digestion kinetics for both protein and lipid digestion can be considered the same for the three repetitions. In other words, sampling a low amount from each digestion reactor was repeatable. For the readily bioaccessible proteins, the independent reactors gave higher (final) values than when sampled from one reactor. Oppositely, in the case of lipolysis, a slightly higher extent was estimated by the model for the single reactor experiment *versus* using eight independent reactors (91% *versus* 81–83%, respectively). This shows that when one samples from a single reactor a (slightly)

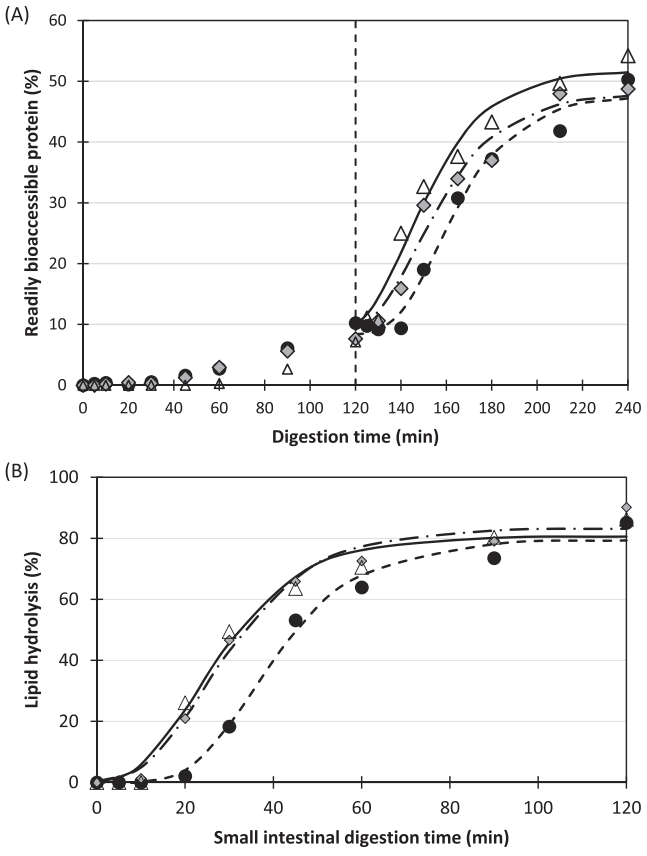

Fig. 3. Time dependent evolution of nutrient hydrolysis (%) quantified as a function of *in vitro* digestion time under semi-dynamic conditions. (A) Release of readily bioaccessible protein and (B) lipid hydrolysis (%) were quantified for Ensure® Plus Vanilla. Symbols represent experimental data (● 20 mL, ◆ 30 mL, and △ 40 mL starting oral volume), while lines represent predicted values of the corresponding Gompertz equation (Eq. (6)). The vertical dashed line in (A) indicates the transition from the gastric to the small intestinal phase.

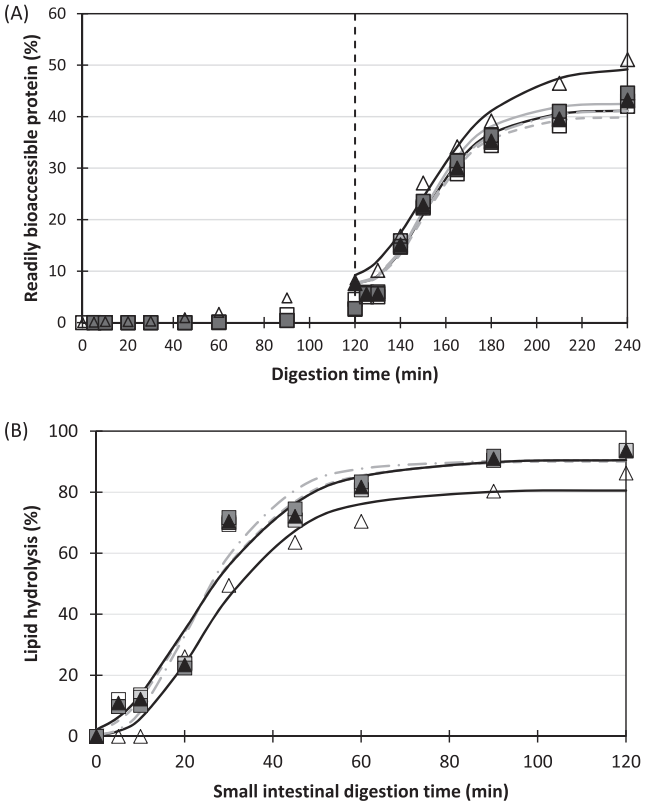

Fig. 4. Time dependent evolution of nutrient hydrolysis (%) quantified as a function of *in vitro* digestion time under semi-dynamic conditions by sampling from one reactor vessel *versus* sampling from eight independent reactor vessels. (A) Release of readily bioaccessible protein and (B) lipid hydrolysis (%) were quantified for Ensure® Plus Vanilla. Symbols represent experimental data (□ repetition a, ■ repetition b, ■ repetition c, ▲ average of the repetitions a, b, and c). For the sake of comparison, △ represents the digestion kinetics when sampled from eight independent vessels when a total volume of 160 mL was reached in the small intestinal phase. Lines represent predicted values of the corresponding Gompertz equation (Eq. (6)) (diverse dashed line styles: the three independent repetitions (a-b-c) when sampled from one vessel, full lines: average digestion behavior of repetitions a, b, and c, and digestion behavior when sampled from eight independent vessels (160 mL SIP)). The vertical dashed line in (A) indicates the transition from the gastric to the small intestinal phase.

different digestion kinetics could be obtained in comparison to using independent reactors and one should be careful when interpreting such data.

3.2.3. Semi-dynamic digestion kinetics of Ensure® plus

From the previous experiments, we obtained kinetic data that we will discuss in more depth in this paragraph. For the gastric phase, three experiments were performed: a static approach, a dynamic SGF addition and thus pH change, and a dynamic approach in which both enzyme and SGF were added as a function of time (Fig. 5). Under static gastric conditions, a rapid increase in readily bioaccessible proteins was observed, which slowed down after 45 min of gastric digestion time (Fig. 5). At the end, around 12% of amino acids and dipeptides were released. Oppositely, for both semi-dynamic conditions, a lag phase was observed after which the release steeply increased until a value around (8%). Due to this digestion pattern in which the bioaccessible protein did not reach a plateau in the gastric phase, these data could not be modelled.

The presence of the long lag phase is explained by the pH conditions in the gastric phase, which decreased from 6.6 to around 2.4 at the end (Fig. 5, Figures A and B, Supplementary Material). The optimal pH of pepsin is around 1.8–2.5, while having an activity of 5–30% at pH values of 3 to 4, and a negligible activity above pH 5 (Kondjoyan, Daudin, & Santé-Lhoutellier, 2015; Pletschke, Naudé, & Oelofsen, 1995). After 45 min, the pH of the chyme drops below 5 and after 75 min of gastric digestion the pH reaches a value of 4. In other words, pepsin starts to be significantly active only after 45 min of gastric digestion, resulting in the observed lag phase. This gradual change in pH is an interesting element as also lingual amylase and gastric lipase are present *in vivo,* having an optimal activity at higher pH values (around 6.8 and 4, respectively) (Carriere et al., 1991; Pedersen, Bardow, Jensen, & Nauntofte, 2002). However, for the simplicity of this proof-of-concept study, we did not include these enzymes. The limited availability of lingual amylase and gastric lipase also largely increases the costs of such digestion experiments. The addition will/should be considered, however, in future experiments aiming to understand effects of, for example, food design on digestion kinetics. No substantial different trends in digestion behavior were noticed for the two semi-dynamic gastric experiments. This means that mainly pH, and not the pepsin amount, was the rate-limiting factor in these pepsin-driven digestion experiments. Additionally, the final amount of readily bioaccessible proteins released, was very similar among the two semi-dynamic digestion conditions studied yet significantly higher for the static case. This implies that static digestion studies could overestimate the final digestibility of protein by simulating digestion for 2 h close to the optimal pH for pepsin activity.

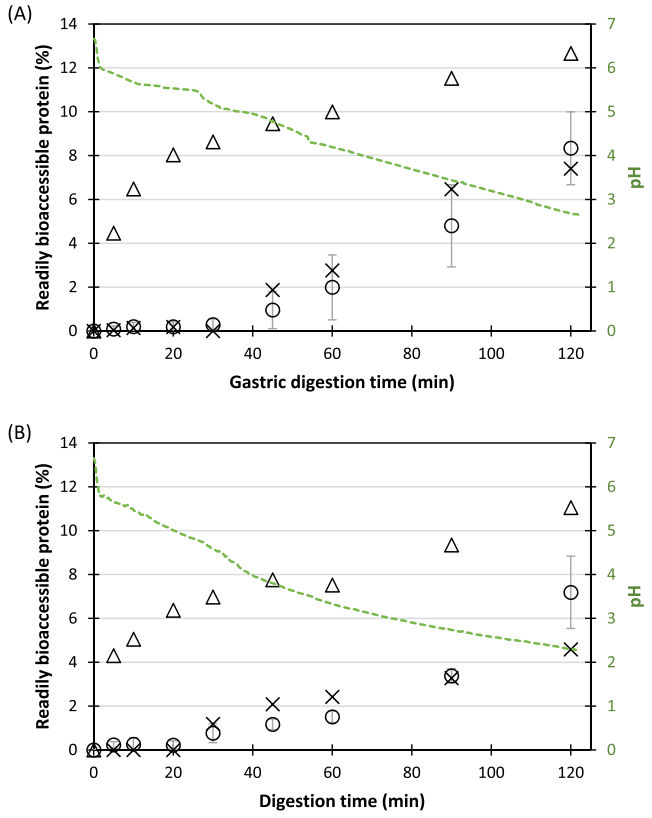

Fig. 5. Time dependent evolution of release of readily bioaccessible protein (%) quantified as a function of *in vitro* gastric digestion time under different digestion conditions. The (A) Ensure® Plus Vanilla drink and (B) cooked lentil samples were digested using a (△) static approach, (×) gradual pH decrease (only dynamic SGF addition), and (○) average of the three repetitions of the semi-dynamic approach in which both enzyme and SGF were added as a function of time. Symbols represent experimental data. The green, dashed line represents the pH profile measured in one of the vessels. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In an independent experiment, we further digested the Ensure® drink in a semi-dynamic small intestinal phase. A similar digestion pattern was observed for protein digestion as in the gastric phase: a lag phase was followed by a steep increase of digestibility. The lag phase was, however, much shorter than in the gastric phase (Fig. 3A). We assume that the lag phase in the small intestine was largely determined by the diffusion of the enzyme towards its substrate and the changing enzyme-to-substrate ratio. A plateau value was not yet reached in these experiments, yet the flattening of the curve is just visible. The estimated extent of readily bioaccessible proteins reached at the end of the small intestinal phase was around 50% which is in line with other studies on milk proteins (Egger et al., 2019). Also, the lipid digestion kinetics were characterized by the same digestion pattern. The gradual addition of both pancreatic lipase and bile salts significantly changed the digestion kinetics compared to the static conditions. The gradual secretion most likely slows down the whole hydrolysis process as lipase first needs to diffuse towards its substrate and bile salts need to prepare the interface for lipase absorption by displacing other emulsifier(s) present (Maldonado-Valderrama, Wilde, Macierzanka, & Mackie, 2011). As shown in a previous study (Verkempinck et al., 2022), bile salts might be most likely the rate-determining factor here as they play an essential role at different levels of lipid digestion. For a more detailed description of the role of bile salts in lipid digestion, we kindly refer to the review paper of Maldonado-Valderrama et al. (2011). The final lipolysis extent was very similar for the static and semi-dynamic digestion approaches (Table 2). This implies that the use of the static INFOGEST protocol gives a good estimation of the final hydrolysis extent of particular nutrients in the small intestinal phase. Additionally, a final lipolysis extent of around 80% was reached, which is line with static studies on low-lipid emulsions (Infantes-Garcia et al., 2022; Salvia-Trujillo, Qian, Martín-Belloso, & McClements, 2013). To the best of our knowledge, this study is the first combining a multireactor kinetic semi-dynamic digestion approach with empirical modelling.

3.3. Cooked lentils

3.3.1. Repeatability of the digestion kinetics using a selected reactor volume

To check the repeatability of the multireactor system in mimicking the semi-dynamic digestion conditions for the cooked lentil sample, we evaluated the digestion kinetics in triplicate using the same reactor volume. We selected the predefined minimum starting volume of 30 mL (Section 3.2.1). The data obtained are shown in Fig. 6 and the estimated parameters are given in Tables 1 and 3. Both for protein and starch digestion, no substantial different trends were observed in terms of hydrolysis kinetics (Table 3). From this, we conclude that the multireactor system allows to study in a repeatable way semi-dynamic

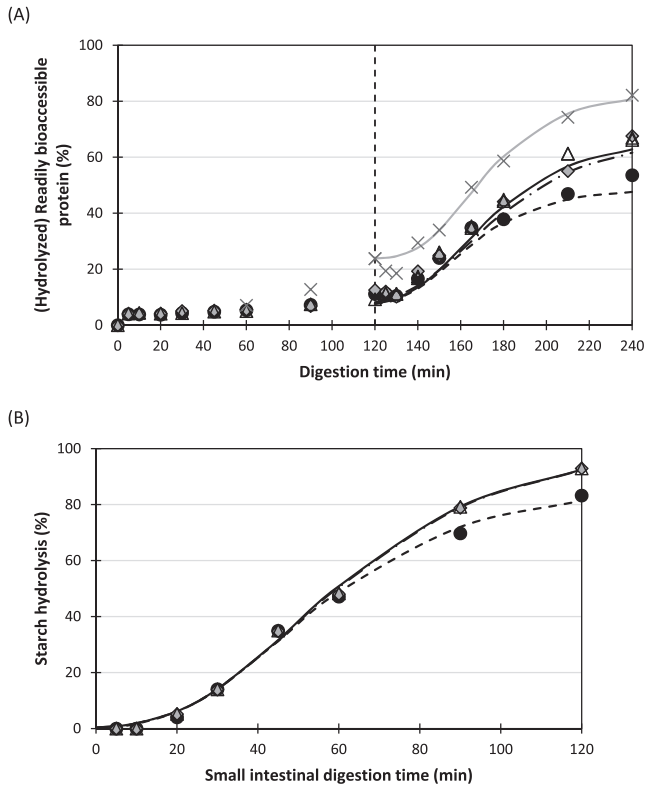

Fig. 6. Time dependent evolution of nutrient hydrolysis (%) quantified as a function of *in vitro* digestion time under semi-dynamic conditions. (A) Release of readily bioaccessible protein and (B) starch hydrolysis (%) were quantified for the cooked lentils. Symbols represent experimental data (● repetition 1, ■ repetition 2, △ repetition 3, and × hydrolyzed readily bioaccessible fraction), while lines represent predicted values of the corresponding modified Gompertz equation (Eq. (6)). The vertical dashed line in (A) indicates the transition from the gastric to the small intestinal phase.

digestion kinetics. The significant differences detected for one repetition were ascribed to deviations that may occur when repeating digestion experiments.

3.3.2. *Semi-dynamic digestion kinetics of a cooked lentil sample*

In similarity to the Ensure® drink, we also performed three gastric phase experiments for the cooked lentils sample: a static approach, a dynamic SGF addition and thus pH change, and a dynamic approach in which both enzyme and SGF were added as a function of time (Fig. 5B). The static condition presented again a fast increase in released readily bioaccessible proteins followed by a less steep trend after 45 min of gastric digestion. The semi-dynamic cases were once more characterized by a lag phase, followed by a rise in protein hydrolysis, and finally a plateau value that was not yet completely reached. In similarity to the Ensure® drink, mainly the gradually decreasing pH determined the digestion behavior in the semi-dynamic gastric phase as discussed before. Also in this case, the static case presented a significantly higher release of readily bioaccessible protein after 2 h of gastric simulation.

The cooked lentil sample was also further digested under semi-dynamic digestion conditions in the small intestinal phase (Fig. 6). In similarity to all other digestion patterns observed under semi-dynamic digestion conditions, a short lag phase was observed before digestion products were formed until a kind of plateau was reached.

For proteins a 12–15 min lag phase was observed before amino acids and small peptides were released until 50 to 64%. Only slight differences were observed among the three repetitions, which mainly became clearer towards the end of digestion. These latter values correspond to a readily bioaccessible$_{\text{hydrolyzed}}$ protein fraction of 82%. The acid hydrolysis these samples underwent, further released free α-amino groups allowing an easier interpretation of the amount of (un)digested protein (Pälchen et al., 2021). This amount is in line with other work performed on pulses at the same research unit (Gwala et al., 2020; Pälchen et al., 2022). Furthermore, this value also indicates that proteolysis is not yet complete, which could also be anticipated as the digestion kinetics did not reach the plateau at the end of the simulation (Fig. 6A).

For starch, a lag phase of around 20 min was observed. This is explained by both the gradual amylase secretion and its subsequently need to find its substrate that is encapsulated by a (porous) cell wall. A delayed initiation of starch digestion and thus a lag phase as a result of barrier properties of the cotyledon cell wall and intracellular matrix was reported previously for cotyledon cells isolated from pulses such as common beans and Bambara groundnuts studied under static conditions (Gwala et al., 2020; Pallares Pallares et al., 2018) and was discussed in more detail by Duijsens et al. (2021). However, under semi-dynamic digestion conditions, this observed lag phase has most likely also a different origin. If we compare the data obtained under static conditions (Fig. 2C and 2D) to the ones obtained under semi-dynamic (Fig. 6A and 6B), we see that the digestion conditions significantly impacted the digestion kinetics, yet not final digestibility. In other words, semi-dynamic digestion conditions significantly impacted the way nutrients were digested, but the static INFOGEST protocol could give a good estimation of the final nutrient digestibility (Tables 1 and 3). A starch hydrolysis of 81–93% was reached after 2 h of small intestinal digestion. This is again in line with previous static studies on diverse pulse types (Edwards, Maillot, Parker, & Warren, 2018; Gwala et al., 2020; Pälchen et al., 2022; Rovalino-Córdova, Fogliano, & Capuano, 2019).

4. Conclusions

In this work, the potential of a custom-made computer-controlled multireactor system (BioXplorer 100, H.E.L Group) was investigated in the field of food digestion simulations (MuReDi). The MuReDi system allows to mimic particular semi-dynamic digestion conditions in multiple reactors. Simulating semi-dynamic *in vitro* digestion conditions is of particular importance when a more realistic evaluation of macronutrient digestion kinetics is the focus. A range of semi-dynamic digestion conditions were tested in preliminar experiments. The ones employed in this work were largely based on the recommendations given in the static and semi-dynamic gastric digestion protocols published by the INFOGEST consortium. We showed that the multireactor can be used from a working volume of 30 mL onwards. Besides, the digestion kinetics were repeatable regardless the starting working volume (when above 30 mL) for both a liquid Ensure® Plus drink and a solid, cooked lentil sample. The application of semi-dynamic digestion conditions, which come closer to human conditions, significantly altered the digestion kinetics of these two food types when compared to static *in vitro* digestion conditions. Both in the gastric and small intestinal phase, the semi-dynamic digestion kinetics were characterized by an initial lag phase, followed by an increased formation of nutrient hydrolysis products until reaching a final (plateau) value. The final digestibility extent was fairly similar between the static and semi-dynamic approach.

By applying such semi-dynamic approaches, we are able to get one step closer to reality which in turn allows to gain more realistic insights into the digestion processes that could take place *in vivo*. Nevertheless, overall costs of the experiment increases, and the overall throughput is reduced. Therefore, static approaches can still be used to screen foods in terms of nutrient hydrolysis extents, particularly in the small intestine, and to compare rates as impacted by food design. It is advisable to select a digestion approach depending on the research question one aims to answer. In future work, the addition of salivary amylase and/or gastric lipase should, for example, be considered in this semi-dynamic kinetic approach. Finally, the increased complexity of (semi-)dynamic types of *in vitro* digestion models demands for a data analysis approach that goes

beyond the commonly used approaches for static *in vitro* data. This can eventually lead to hybrid strategies, joining *in vitro* and *in silico* approaches, to more accurately translate *in vitro* observations into *in vivo* predictions (Le Feunteun et al., 2021).

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CRediT authorship contribution statement

S.H.E. Verkempinck: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft. **D. Duijsens:** Data curation, Formal analysis, Investigation, Writing – review & editing. **D. Michels:** Data curation, Formal analysis. **J.M. Guevara-Zambrano:** Data curation, Formal analysis. **M.R. Infantes-Garcia:** Data curation, Writing – review & editing. **K. Pälchen:** Conceptualization. **T. Grauwet:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2022.111301.

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