Supplementary Material

Supplementary Material 1: Examples of vessel types and corresponding 3D printing stirrers.

Vessel type	Order number
Titration vessel with thermostat jacket / 5-70 mL, Metrohm	6.1418.150
Titration vessel with thermostat jacket / 50-150 mL, Metrohm	6.1418.250

Two examples of the paddle stirrer that can be used in the semi-dynamic model are presented below.

The 3D printing files for making the stirrers are attached with the file names “3D file Stirrer for smaller Metrohm vessel 5 to 70mL” and “3D file Stirrer for smaller Metrohm vessel 50 to 150mL”. These can be modified depending on the characteristics of the vessel that is available.

Supplementary Material 2: Protocol for pH test tube.

1) Previously warm the eSSF and eSGF stock solutions to 37ºC.
2) Prepare the SSF: add the necessary volume of 0.3 mol/L $CaCl_2(H_2O)_2$ to achieve 1.5 mmol/L and purified water to obtain the require concentration of SSF.
3) Add X mL of SSF to the Y g of food. Where X corresponds to the dry weight of the Y mL of sample. For clarity, X is for now on assumed to be 3 mL and Y is assumed to be 20 mL.
4) Incubate for 2 minutes at 37ºC with continuous mixing.
5) Prepare the SGF: add the necessary volume of 0.3 mol/L $CaCl_2(H_2O)_2$ to achieve 0.15 mmol/L $CaCl_2(H_2O)_2$ and purified water to obtain the required concentration of SGF.
6) Add the SGF without enzymes using a 1:1 volume ratio with the oral mixture (23 mL in this example) and check the pH. Slowly titrate using 1 mol/L HCl (or of higher molarity if the volume needed is abundant, e.g. > 10% of the SGF volume, 2.3 mL in this example) until the pH reaches 2. Take note of the volume and concentration used to reach pH 2. These are indicative of the necessary amount of HCl to add during the gastric phase.

Supplementary Material 3: Excel spreadsheet.

This Excel file provides the calculations needed to generate all the parameters for conducting the semi-dynamic digestion. File name “Semi-dynamic model protocol”

Supplementary Material 4: Digestion step by step procedure

This procedure consists of three consecutive phases: oral, gastric and small intestinal digestion. The same example as described in the main paper will be used for convenience (Table 3). This is, 20 mL of a food sample, which has 3 mL of dry weight and 0.8 kcal/mL, 2 mL of 1.5 mol/L HCl is needed to reach pH 2 and five gastric emptying aliquots are taken to simulate the emptying. The rest of the parameters can be calculated using the Excel spreadsheet provided.

Oral phase: 1:1 (v/v) final ratio of the dry weight of the food to SSF (eSSF + enzyme + water + $CaCl_2(H_2O)_2$)

Note that the oral phase should always be included (even for liquid food samples) although the amylase is optional in the absence of starch.

1) Previously warm the eSSF solution.
2) Take 20 mL of food and warm it up to 37ºC.
3) If needed, simulate mastication by mincing it in a mincer with either a 3 mm grid. Depending on the food (e.g. bread), mincing can be done together with some water or eSSF to obtain a consistency similar to tomato paste or mustard.
4) Prepare α-amylase solution in purified water, in the concentration required to accomplish the next step. Keep the solution on ice.
5) Prepare SSF by adding the calculated amounts of eSSF, α-amylase in order to achieve a final activity of 150 U/mL of SSF volume, $CaCl_2(H_2O)_2$ in order to achieve 1.5 mmol/L in the final mixture and purified water. In the example, 2.40 mL eSSF, 0.015 mL $CaCl_2(H_2O)_2$ (0.3 mol/L), 0.435 mL water and 0.15 mL of amylase solution.
6) Add the 3 mL of SSF to each 20 mL of sample
7) Incubate for 2 minutes at 37ºC with shaking.

Gastric phase: 1:1 (v/v) final ratio of oral content to SGF (eSGF + enzyme + HCl + water + $CaCl_2(H_2O)_2$)

1) Previously warm the eSGF solution to 37ºC.

2) Prepare enzyme solutions in eSGF. Prepare the concentration needed to achieve a final activity of 4,000 U/mL for pepsin and 120 U/mL for gastric lipase in the SGF volume. Keep the solutions on ice.
3) Prepare the basal solution. This contains the 10% of the SGF. In the example, the total basal volume is 2.3 mL containing 1.61 mL eSGF, 0.001 mL $CaCl_2(H_2O)_2$ (0.3 mol/L), 0.23 mL enzyme solution, 0.20 mL HCl (1.5 mol/L) and 0.259 mL water. This solution is placed in the reaction vessel.
4) Prepare the simulated gastric electrolyte mixture, which contains the 90% of SGF except the enzyme solution. In the example, the total volume is 18.63 mL, which contains 14.49 mL eSGF, 0.009 mL $CaCl_2(H_2O)_2$ (0.3 mol/L), 1.8 mL HCl (1.5 mol/L) and 2.33 mL water.
5) Set the rate of the pumps to deliver the simulated gastric electrolyte mixture (18.63 mL in this example) and, the pepsin and gastric lipase solutions (2.07 mL in this example) over the gastric digestion time (200.5 min in this example).

    5.1) The simulated gastric electrolyte mixture will be delivered in a rate according to the total digestion time. In this example, 18.63 mL/200.5 min = 0.0929 mL/min.
    5.2) The 90% of enzyme solution will need a flow rate calculated as follows: 90% enzyme volume / total gastric digestion time. In this example, 2.07 mL of enzyme solution is added gradually, which will need a flow rate of 0.010 mL/min for 200.5 min.

6) Place the simulated gastric electrolyte gastric mixture and enzymes solutions in the pumps. The latter should be kept on ice, in separate delivery system.
7) Connect all the delivery systems to the vessel lid using plastic tubing. All the tubes are preferably placed in the bottom of the vessel being inside the digesta and preferentially on the vessel walls. This will avoid the discontinuous flow of the secreted fluids.
8) Set the mixing system that is more appropriate to the tested meal.
9) Add the bolus obtained from the oral phase in the reaction vessel.
10) Place the pH probe in the vessel using one of the openings of the vessel lid (if applicable) and cover the rest of openings with stoppers or parafilm® to get a closed system.

11) Start the delivery of the simulated gastric electrolyte gastric mixture and enzymes solution at the same time, as well as using a timer in order to take the aliquots for the emptying.
12) The emptying is performed manually by removing the corresponding volume at the corresponding time. Place the sample in an appropriate tube and adjust the pH to 7.0 by addition of NaOH. Then snap freeze the sample with liquid nitrogen, if there is no damage in the structure of the samples, for the intestinal phase to be done on a separate day. Take note of the NaOH volume added since it is taken into account for the calculations of the intestinal phase.

Intestinal phase: 1:1 (v/v) final ratio of emptied digesta from gastric phase to SIF (eSIF + enzyme + bile + water + $CaCl_2(H_2O)_2$ + NaOH added to reached pH 7 at the end of the gastric phase).

The intestinal phase will not be dynamic (i.e. there is no absorption and all enzymes and fluids are added at the beginning). However, a separate intestinal phase will be performed for each gastric emptying aliquot. It is advisable for the intestinal digestion to be performed in the same tube which was collected from the gastric phase.

Choose procedures a) or b) if the intestinal phase will be performed with pancreatin or individual enzymes, respectively:

a) Prepare the pancreatin suspension in eSIF in order to reach 200 U/mL of trypsin activity in the SIF volume. Additional pancreatic lipase may be needed for the digestion of lipid containing food (to reach the required lipase activity, consider possible trypsin activity present in the pancreatic lipase). Keep in ice.
b) Prepare the porcine trypsin, the chymotrypsin, the porcine pancreatic α-amylase, the porcine pancreatic lipase and the co-lipase solutions in eSIF, in order to reach 200 U/mL, 50 U/mL, 400 U/mL, 4000 U/mL, 2:1 ratio, respectively, in the SIF volume. Keep in ice.

1) Previously warm the eSIF stock solution to 37ºC.
2) Prepare the bile solution in eSIF in order to reach the concentration of 20 mmol/L of bile salts of SIF. Bile solubilisation can be done on a rotating wheel at 37ºC for at least 30 min or overnight at room temperature.

3) Check the pH and take note of any addition of pH adjustment volume. Take into account the volume of NaOH added in each sample to stop pepsin activity.
4) Add $CaCl_2(H_2O)_2$ (0.3 mol/L) in order to reach 0.6 mmol/L in the final mixture.
5) Add bile solution to reach 20 mmol/L in SIF.
6) Add enzyme solution according to options (a) or (b).
7) Add purified water in order to achieve the required concentration of the SIF.
8) Incubate the samples on a rotating wheel or a shaking incubator at 37°C for 2 hours.