

Experiment:



Date:

Protocol *in vitro* digestion – INFOGEST 2.0

For 7 Samples

PREPARATIONS

1. Fill the Styrofoam box with ice
2. Get all the simulated digestive solutions, CaCl_2 , refill the MiliQ water
3. Weigh RGE and put it on ice
 - a. RGE (42.35 mg):
4. Turn on dry bath and set the temperature to 39.7°C -> press start
5. Install the temperature sensor from the pH meter in a tube with water and wrap it with parafilm
6. Warm up the pH buffers
7. While the pH buffers are warming up, prepare the excel sheet (option to weigh the enzyme here)
8. Calibrate the pH meter
9. Set dry bath to 550 SV

ORAL DIGESTION

1. Add 400 μL SSF to S1-S7
2. Add 3 μL CaCl_2 to S1-S7
3. Add the MiliQ water according to the excel to S1-S7
4. Vortex (avoid turning tube upside down)
5. Incubate for 2 mins

GASTRIC DIGESTION

1. Add 800 μL SGF to S1-S7
2. Adjust the pH to 3 \pm 0.2
3. Add the remaining amount of MiliQ water to S1-S7 according to the excel
4. Dissolve RGE in 800 μL MiliQ and add 100 μL of it to all of the tubes
5. Start the timer for 2h
6. Re-adjust the pH after 10 and 60-75 minutes
7. During this time: lunch break, cleanup
8. After the second pH adjustment, pre-weigh the enzymes pancreatin and bile
 - a. Pancreatin (480 mg):
 - b. Bile (164.38 mg):
9. Dissolve Bile in 2.250 mL SIF and put it in the water bath but keep pancreatin on ice

INTESTINAL DIGESTION

1. Add 850 μL SIF to S1-S7
2. Add 4 μL CaCl_2 to S1-S7
3. Adjust the pH to 7 \pm 0.2
4. Add the remaining amount of MiliQ water to S1-S7 according to the excel
5. Dissolve Pancreatin in 4.5 mL SIF
6. Add 250 μL bile to S1-S7
7. Add 500 μL pancreatin to S1-S7
8. Start the timer for 2h
9. Re-adjust the pH after 10 and 60-75 minutes
10. During this time: pre-label tubes for supernatant and full digesta and pre-cool the centrifuge in D31

CENTRIFUGE:

1. Transfer 1.5 mL of S1-S7 to the pre-labeled 2.5 mL Eppendorf tubes for the full digesta. Make sure to clean the pipette between each tube.
2. Centrifuge the remaining tubes at $10'000\text{ g}$, 4°C , for 30 minutes
3. During this time: cleanup and preparation to store the tubes
4. After centrifuge: transfer the supernatant to the 15 mL pre-labeled conical tubes, tape and label everything and store it in a plastic bag in the -20°C