

Enzymatic predigestion of fermented whey to assess its impact on gut microbiota using *in vitro* digestion models

Grasse, M.¹, Aguilar Trujillo, N.¹, De Martinis, C.², Gonza-Quito, I.¹, Katsandegwaza, B.¹, Delcenserie, V.¹
¹Department of Food Sciences, FARAH - Veterinary Public Health, University of Liège, B43b, 4000 Liège, Belgium
²Faculty of Animal Science and Food Engineering - FZEA / USP, University of São Paulo, Pirassununga - SP, 13635-900, Brazil

Scientific context

Part of the ALAFERM project (Interreg Greater Region, EU co-funded), this research contributes to the valorization of agri-food by-products and the sustainability of the dairy sector. Project duration: January 2026 – December 2028.

Whey-derived proteins and fermented dairy components can modulate gut microbiota composition and support intestinal health [1,2].



Whey (by-product of cheese production)

EU: 50 000 000 m³/year

Environmental and economic concerns within the dairy sector.

Solution



Nutritional supplement; fermented whey containing pre- and probiotics

Impact

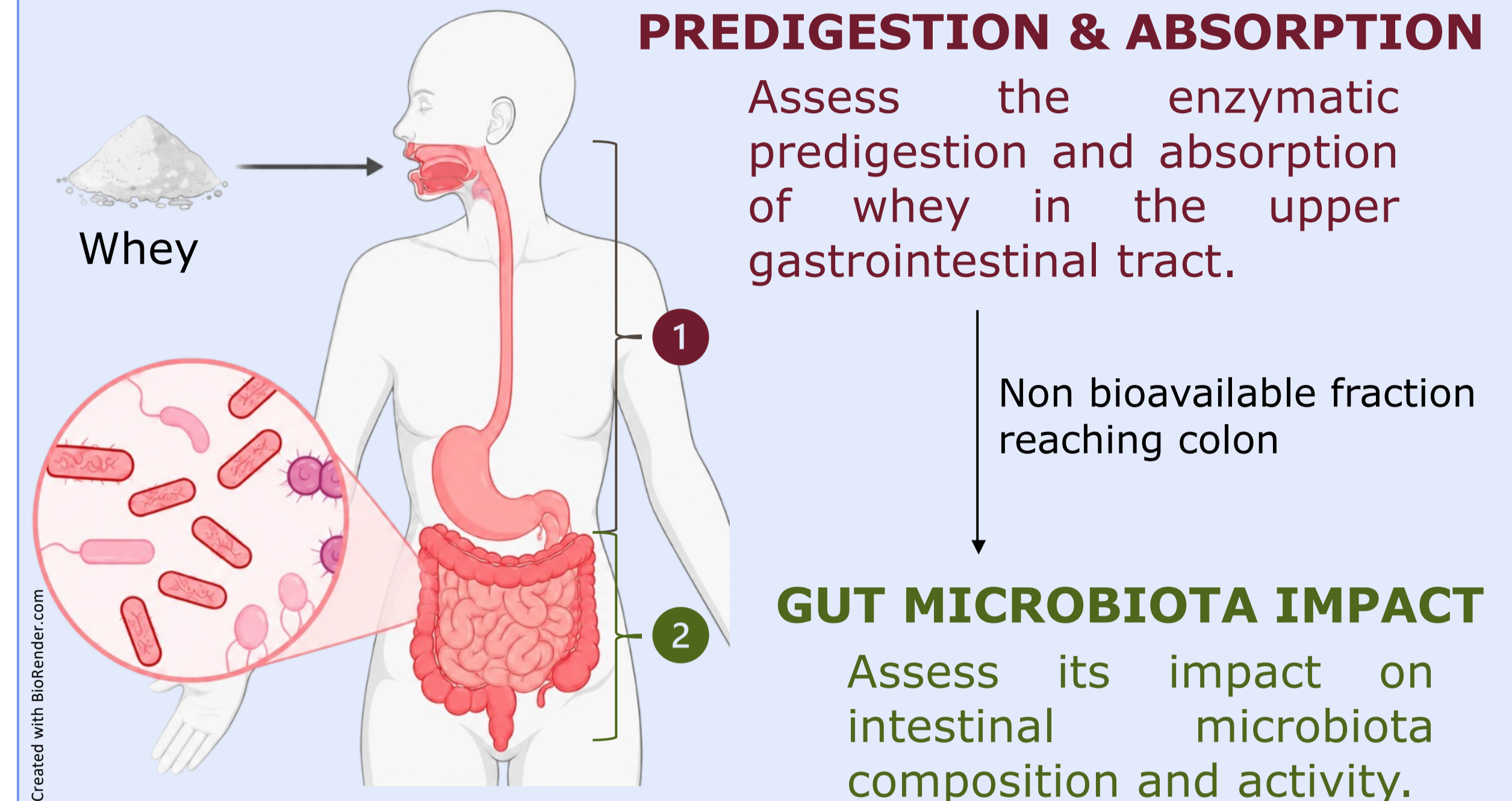
- ✓ Reducing waste in the dairy sector
- ✓ Optimizing resource use
- ✓ Developing a product with potential benefits for gut health



→ **Need to assess the impact of fermented whey on the gut microbiota.**

Goal & Strategy

Assess the effects of this fermented whey containing pre- and probiotics on the gut microbiota.



→ *In vitro* digestive models : INFOGEST 2.0 + M-SHIME® [3,4]

Experimental methods

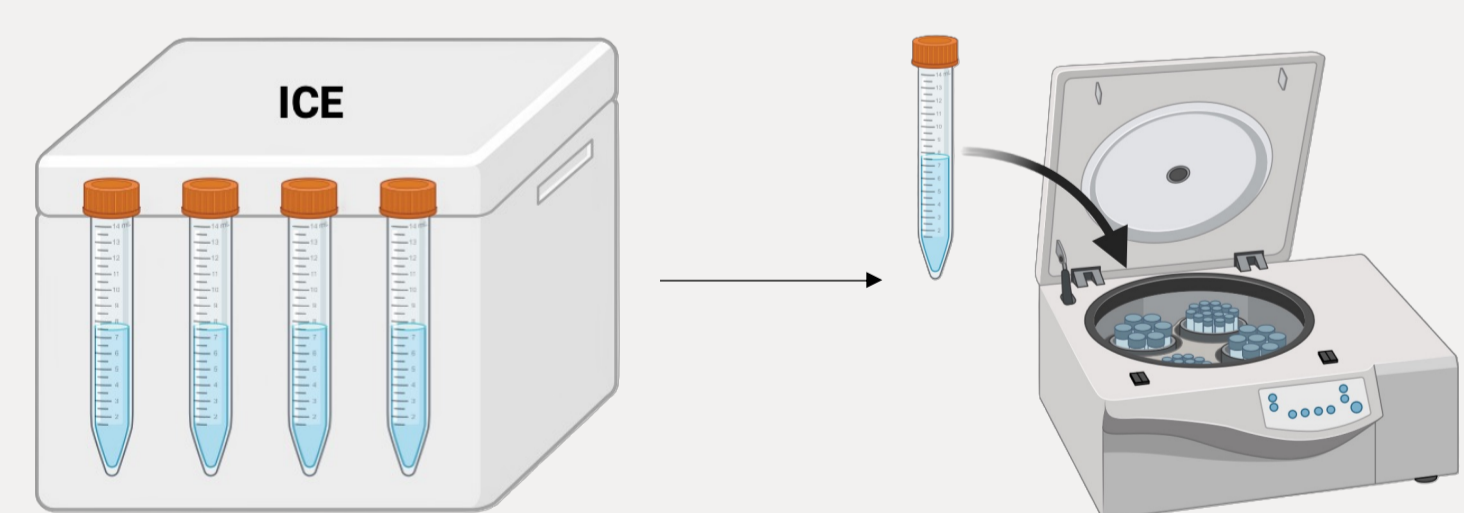
1 Standardized *in vitro* digestion (INFOGEST 2.0) + adaptations fermented whey-specific

- Oral phase**
- Mix Food with simulated salivary fluid (SSF) (1:1 (wt/wt))
 - Include CaCl₂ (1.5 mM in SSF)
 - Add salivary amylase, if necessary (75 U/mL) (no starch)
 - Incubate while mixing (2 min, 37 °C, pH 7)

- Gastric phase**
- Mix oral bolus with simulated gastric fluid (SGF) (1:1 (vol/vol))
 - Include CaCl₂ (0.15 mM in SGF)
 - Add gastric lipase, pepsin (60, 2000 U/mL) (no lipids)
 - Incubate while mixing (2 h, 37 °C, pH 3)

- Intestinal phase**
- Mix gastric chyme with simulated intestinal fluid (SIF) (1:1 (vol/vol))
 - Include bile (10 mM bile salts)
 - Include CaCl₂ (0.6 mM in SIF)
 - Add pancreatin (trypsin activity 100 U/mL)
 - Incubate while mixing (2 h, 37 °C, pH 7)

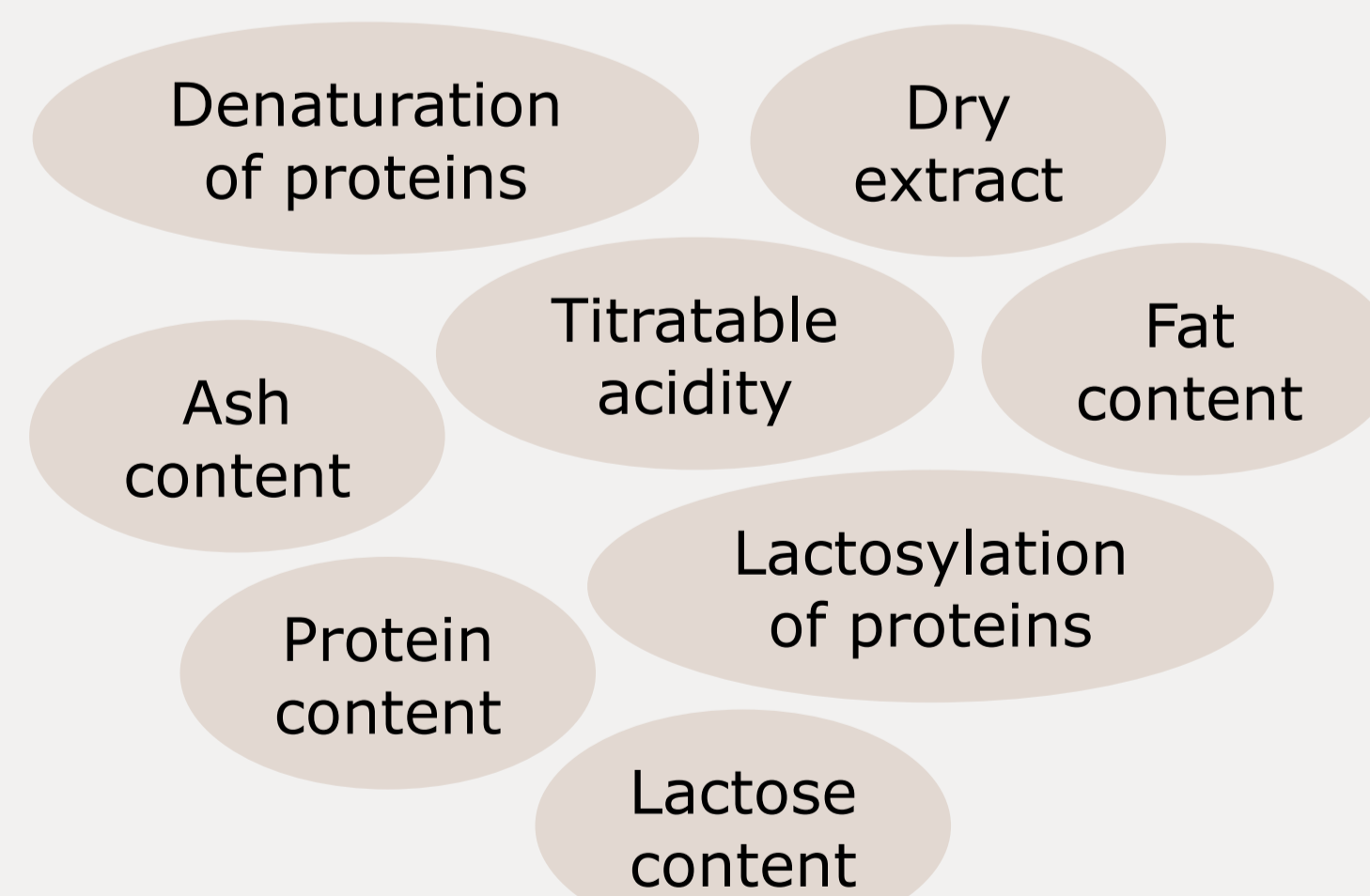
2 Sampling & Sample preparation



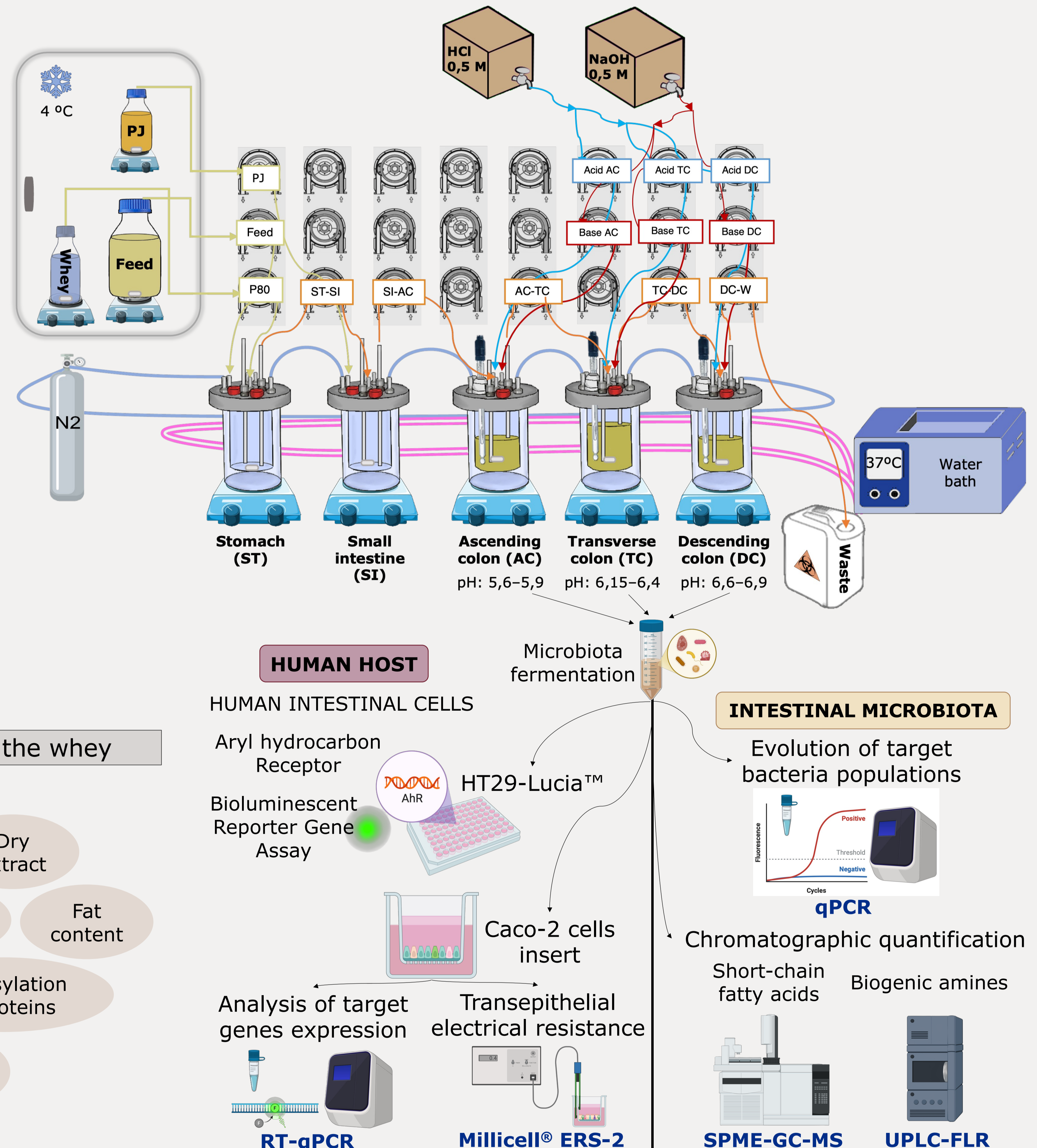
4 °C, 20 min
10000 x g

- Final substrate mixture :
- 95% insoluble fraction
 - 5% soluble fraction (to be confirmed)

3 Characterization of the whey



4 M-SHIME® + analyses



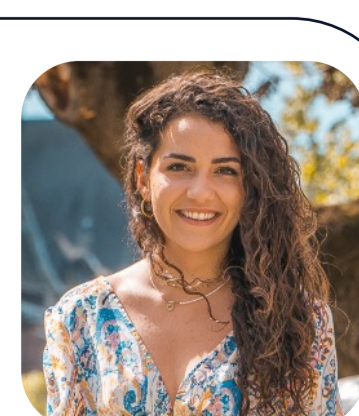
Expected results

- The INFOGEST model is expected to generate a physiologically relevant predigested whey fraction representative of the material reaching the colon.
- This colonic fraction will subsequently be used in the M-SHIME® system to investigate microbiota activity and the production of microbial metabolites, including short-chain fatty acids and other bioactive compounds.
- Intestinal cell models will be employed to evaluate host responses to microbiota-derived metabolites, providing further insight into food-microbiota-host interactions and their potential implications for intestinal health.

References

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3. Brodkorb, André, Lotti Egger, Marie Alminger, et al. 'INFOGEST Static *In Vitro* Simulation of Gastrointestinal Food Digestion'. Nature Protocols 14, no. 4 (2019): 991-1014. <https://doi.org/10.1038/s41596-018-0119-1>.
4. Goya-Jorge, Elizabeth, Irma Gonza, Pauline Bondue, et al. 'Unveiling the Influence of a Probiotic Combination of Heyndrickxia Coagulans and Lactocaseibacillus Casei on Healthy Human Gut Microbiota Using the TripleSHIME® System'. Microbiological Research 285 (August 2024): 127778. <https://doi.org/10.1016/j.micres.2024.127778>.

Contact



Manon Grasse
manon.grasse@uliege.be



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