Effect Of Ultra-Filtration After *In Vitro* Digestion Of Sustainable Food Proteins On SCFA Production By Microbiota

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Background

Proteins from animal- and plant/fungal-derived sources will be degraded differently in the GI-tract. This influences the absorption of the degradation products in the GIT. During passage along the small intestine, amino acids and peptides from highly digested protein will be absorbed by the intestinal cells and will not reach the colon. However, proteins that are not or partly hydrolysed will pass into the colon and serve as a substrate for proteolytic activity or fermentation by its microbiota.

Objective

Analyse the effect of ultra-filtration (UF) on the SCFA production by microbiota compared to fermentation of whole digests.

Method

Animal- and plant/fungal-derived proteins were *in vitro* digested according to an adapted INFOGEST static consensus protocol. The digests were ultra-filtrated using a disk membrane with 1 kDa cut off. The retentate was washed 3 times with buffer after which it was used for mini-fermentation experiments with human microbiota. UF retentate and whole digestions were fermented by the same distal colon microbiota, stabilised by the SHIME system. During the fermentation pressure, SCFA and pH were measured, fig 1.

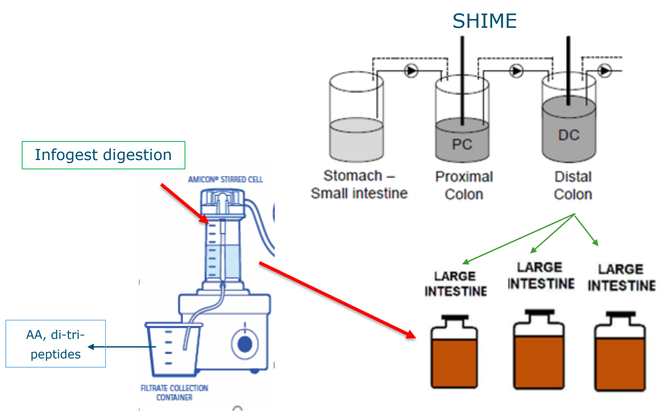

Figure 1. In vitro digestion, ultra filtration and mini-fermentation set up

Results

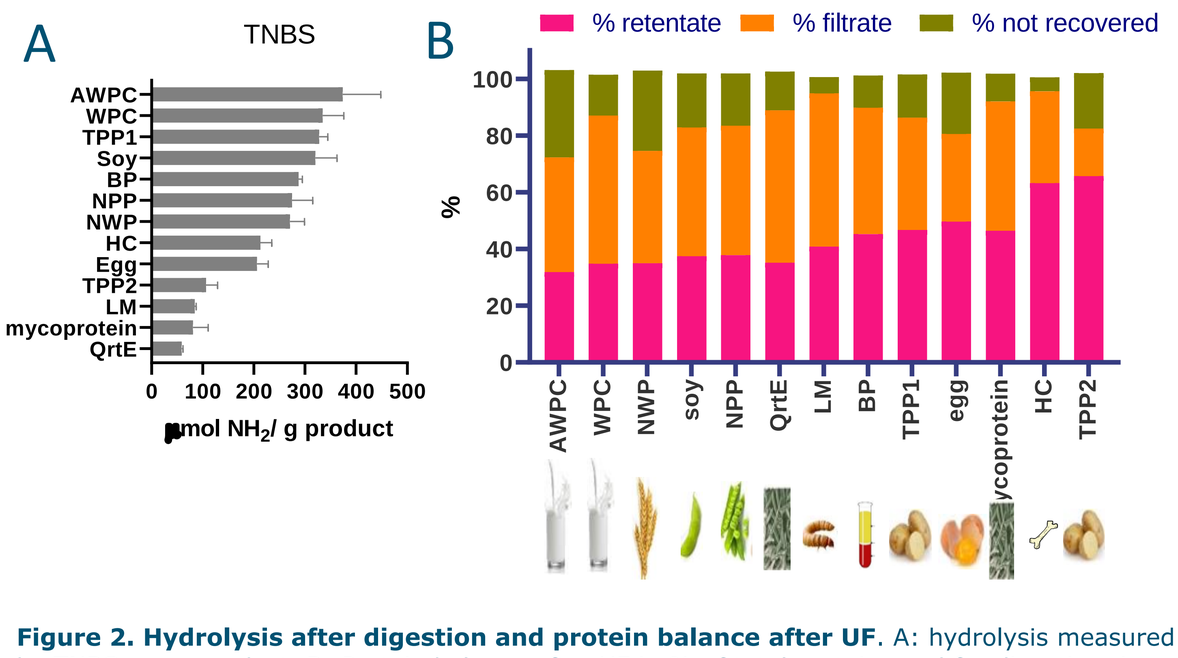

Figure 2. Hydrolysis after digestion and protein balance after UF. A: hydrolysis measured by TNBS per g product. B: Protein balance after UF in % of total protein used for digestion, in pink the retentate which is used for the microbiota fermentation, in orange the proteins that are in the filtrate and in green the proteins that were not recovered.

Results

In vitro digestion:
Differences in degree of hydrolysis comparing the different sources were observed as well as differences in protein amounts that were retained after UF in the retentate (fig 2). Proteins that were hydrolysed had less protein in their retentate as can be seen for both whey proteins, UF on proteins with lower hydrolysis had a higher protein content in the retentate like TPP2.

Mini-fermentation:
The production of total SCFA was higher in whole digests compared to retentate, although this varied among individual SCFA like propionic acid. Comparing whole digest and retentate from the plant dataset, the acetic acid, butyric and iso-valeric acid levels were significantly lower in the retentate. In the animal dataset, only iso-valeric acid was lower in the retentate, fig 3.

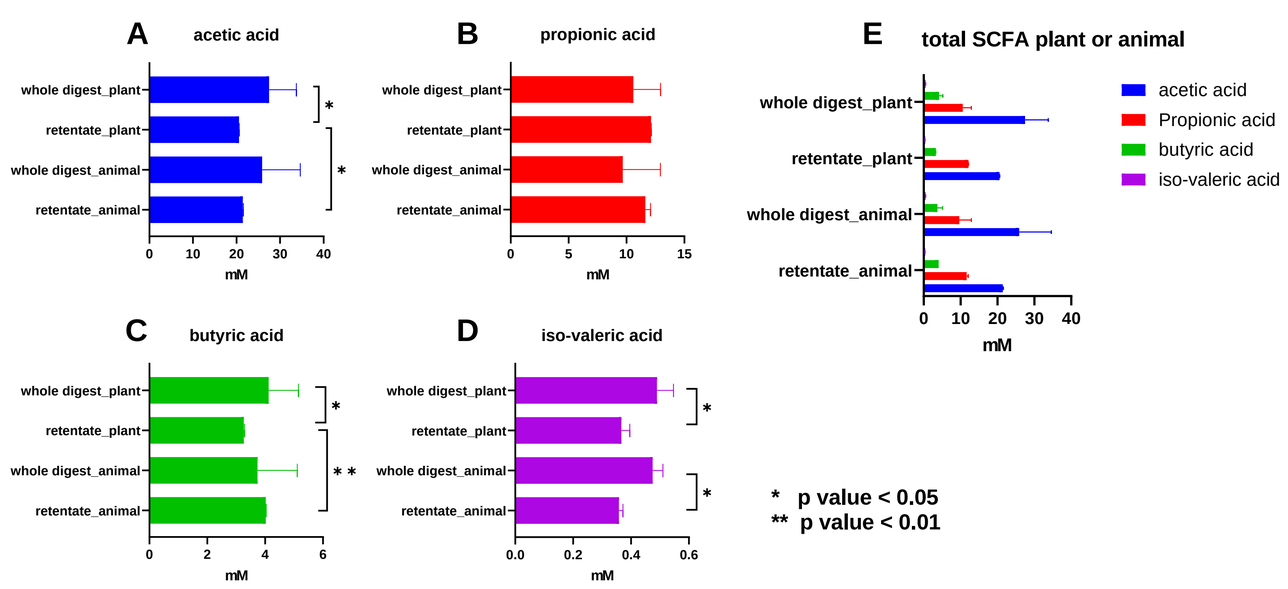

Figure 3. comparison whole digest vs retentate SCFA after 24 h incubation with microbiota. Statistical significance was calculated using the Mann-Whitney Test.

After UF, microbiota produced more acetate and butyrate from animal than from plant/fungal sources if both retentate datasets were compared, fig 3 A and C. Although the amounts of proteins added to the microbiota were lower in the retentate this did not correlate with the amount of total SCFA formed (data not shown).

Conclusions

- UF has an effect on SCFA production. Whole digests gave an overestimation of the total SCFA production.
- Retentate from both plant and animal sources produced more propionic acid than whole digests, although this difference was not significant.

Acknowledgements

Research was co-financed by the SFP-consortium (TKI-AF 15269) in collaboration with BASF, Cargill, Coöperate AVEBE U.A., Darling Ingredients, Lesaffre, Marlow Foods, PepsiCo, Roquette, Mimetas B.V., Nutricia Research, Proti-Farm R&D B.V. and University of Utrecht. The views expressed in this manuscript are those of the authors and do not necessarily reflect the position or policy of the collaborators.

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