

# In vitro impact of *Tenebrio molitor* insect flour on human gut microbiota

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## Introduction

Human diet is in constant change in parallel with society evolution. Modern dietary research seeks not only for new nutritive and safe food sources, but also for those with bioactive potential such as the *Tenebrio molitor* insect flour (TMIF). The nutritive effect of a bioactive ingredient can be evaluated by its effect on gut microbiota modulation, hence *in vitro* models, within their limitations, can provide information about the effect of this ingredient in the microbiota groups and metabolic activity. An important perspective can then be obtained by investigating the hosts gut microbiota, which influences the individual's health state. The understanding of host-microbiota-food component interactions is of major significance, and for that, simulation models can help comprehend that complex relationship. This work aimed the evaluation of the impact of digested and undigested TMIF, at the gut microbiota level. Also deriving from the collected data, it was studied the relevance of TMIF as a safe and nutritional balanced food source, with benefits for the human gut microbiota.

**Table 1-** Nutritional composition of *Tenebrio molitor* insect flour (TMIF) (per 100g).

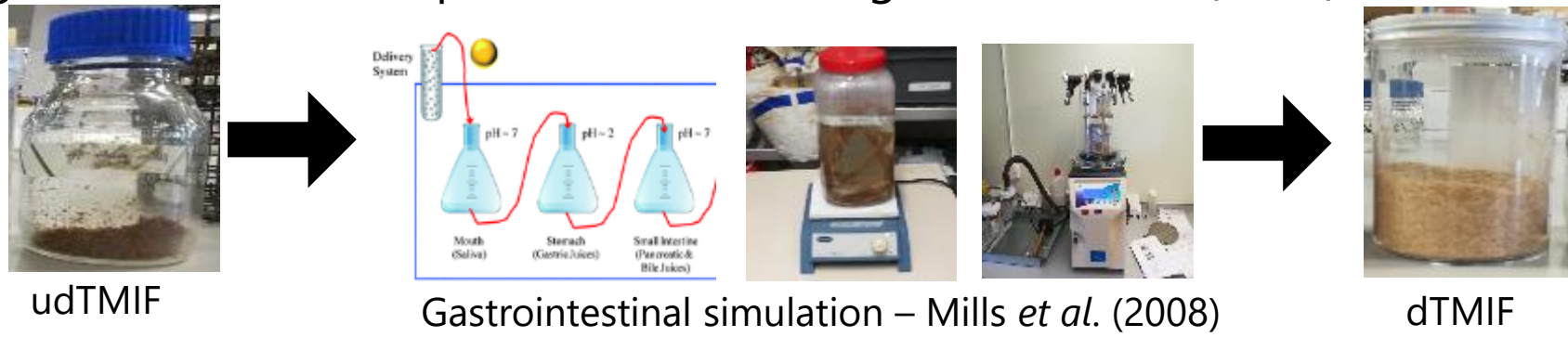
Component	Concentration
Total sugar	<0.10 g
Amino acids (ash)	5.4 g
Cholesterol	0.002 mg
Fiber	3.0 g
Fat	39.4 g (saturated- 8.6 g)
Carbohydrates	<0.10 g
Humidity	7.5 g
Protein	44.6 g
Sodium	142 mg
Energetic value	539 Kcal ⇔ 2242 kJ

dTMIF- digested TMIF; udTMIF- undigested TMIF

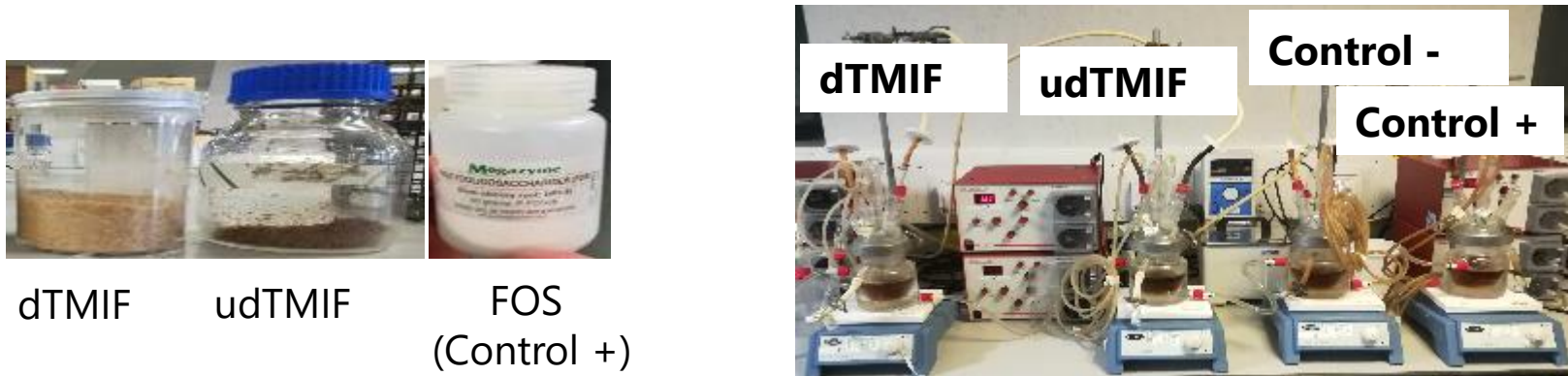
## Material & Methods

The TMIF sample was sterilised at 100 °C for 24 h to guarantee that TMIF was free of microorganisms.

Digestion of TMIF was performed according to Mills *et al.* (2008).



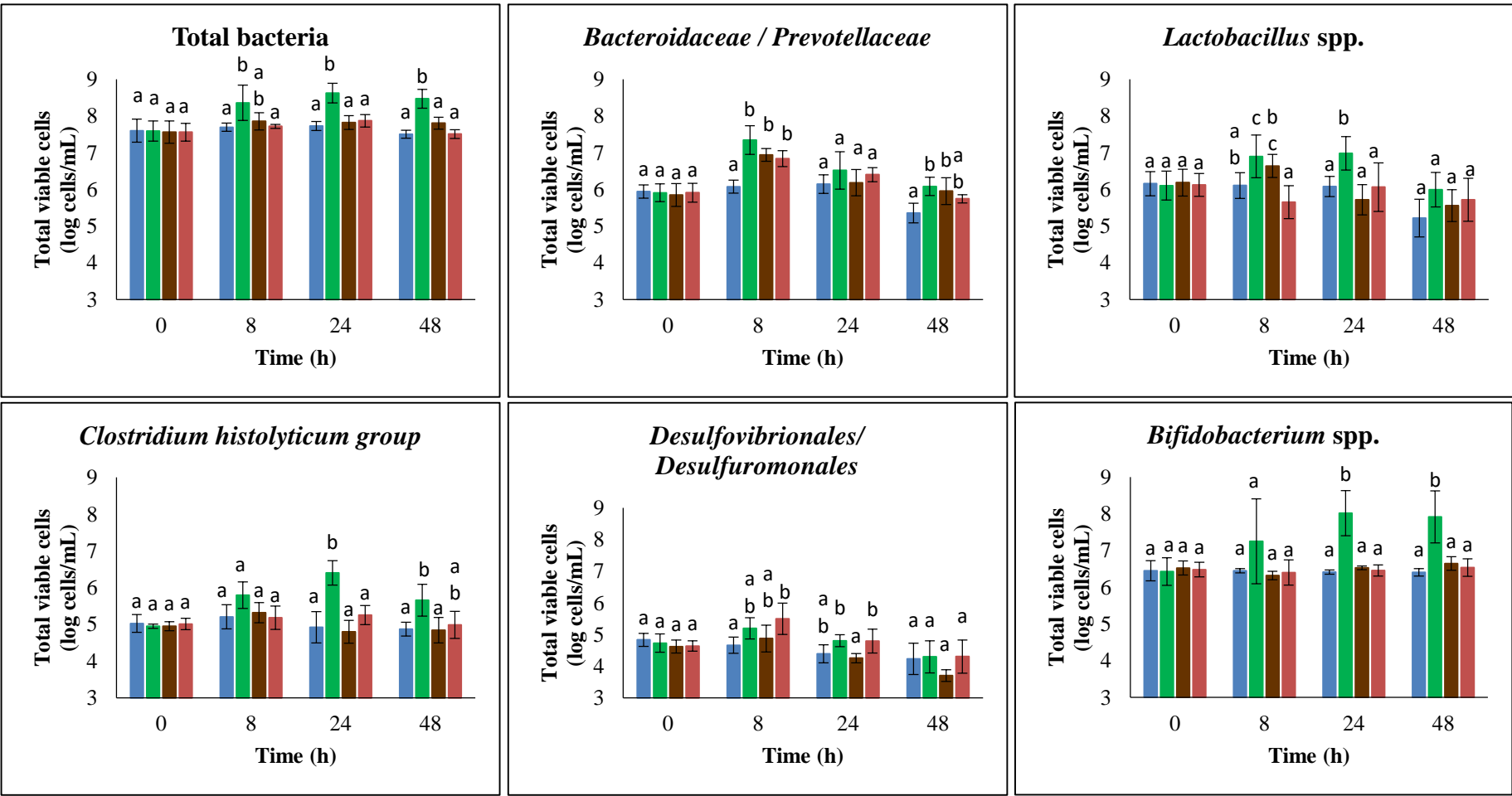
The *in vitro* faecal model was performed according to Rodrigues *et al.* (2016) with slight modifications. Five independent fermentation experiments were carried out. The volunteers had a normal omnivorous diet and had not ingested any antibiotics or other medicines known to affect the microbiota for at least 6 months. Volunteers were 2 males and 3 females aged 22-37 years. Four stirred pH-controlled batch fermenters (pH 6.7-6.9) were run in parallel. Four different conditions were studied in each different vessel: 1) 1% (w/v) dTMIF; 2) 1% (w/v) udTMIF; 3) 1% (w/v) FOS (Megazyme, Bray, Ireland) as positive control; 4) no carbon source added as negative control.



Batch cultures were running under anaerobic conditions at 37 °C during 48 h, in which 5 mL samples were collected from each vessel at 0, 4, 8, 24 and 48 h for bacterial enumeration by fluorescence *in situ* hybridization combined with flow cytometry (FISH-FCM) according to Grimaldi *et al.* (2017), analysis of SCFA and BCFA by gas chromatography (GC) and quantification of ammonia production with 53659-FluoroSelect™ Ammonia Kit (Sigma-Aldrich, Gillingham, UK).

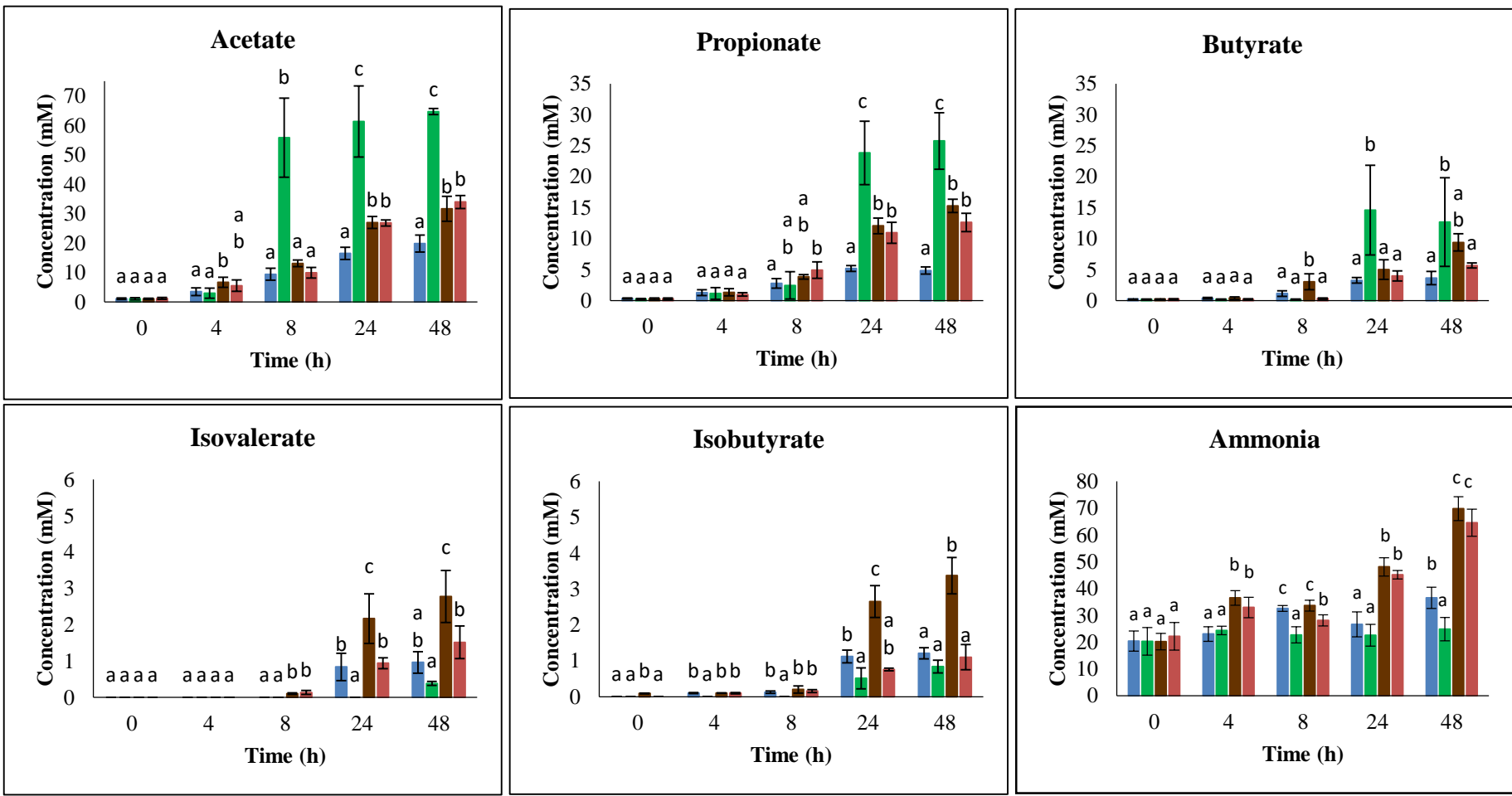
## Results

TMIF promoted the growth of *Bacteroidaceae/Prevotellaceae*, main producers of propionate, and are positively associated with isovalerate and isobutyrate, which for their turn are negatively correlated to blood levels of triglycerides. The growth of bacteria with negative effect on human health (*C. histolyticum* group and *Desulfovibrionales/Desulfuromonales*) were not promoted in TMIF samples. TMIF increased the production of BCFA and SCFA, especially acetate and propionate, which are associated to health benefits (e.g. reduction of appetite and cholesterol). In the TMIF samples, ammonia production was within non-cytotoxic concentration.



**Figure 1-** Bacterial populations (log (cells/mL), means ± SD) detected by FISH-FCM in the faecal samples. Different letters mark statistically significant (p<0.05) differences between samples at each sampling point.

■ Negative control ■ FOS ■ udTMIF ■ dTMIF



**Figure 2-**Concentration (mM, means ± SD) of the metabolic products produced along fermentation time in the faecal samples. Different letters mark statistically significant (p<0.05) differences between samples at each sampling point.

## Conclusions

The used *in vitro* gut microbiota model allowed to assess the impact of TMIF, showing positive effect on the growth of beneficial bacteria of gut microbiota without promoting the growth of bacteria with negative impact on human health. TMIF also promoted the production of SCFA and BCFA, especially on the production of acetate and propionate. The ammonia production in the presence of TMIF was within concentration levels that do not have cytotoxic effects. This model allowed to conclude that TMIF has potential beneficial impact upon the human gut microbiota, and therefore in health.

## References

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