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Impact of novel clean label ham formulations on the human gut microbiota

Teresa Bento de Carvalho[1], Joana Bastos Barbosa[1*], Nelson Mota de Carvalho[1], Carla Souza[1], Célia Costa[1], Norton Komora[2], Alexandra Azevedo[2], Ana Raquel Madureira[1], Paula Teixeira[1]

[1] Universidade Católica Portuguesa, CBQF—Centro de Biotecnologia e Química Fina—Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal
[2] R&D Department, Primor Charcutaria Prima – S.A., Avenida Santiago de Gavião, nº 1142, Gavião, 4760-003, Vila Nova de Famalicão, Portugal.

** e-mail jbarbosa@ucp.pt*

INTRODUCTION

The influence of diet on the human gut microbiota has been the subject of much debate, particularly in relation to innovative products that are still considered novel. Dietary habits have a fundamental impact on the human gut microbiota, which explains the variations observed between individuals and over the course of a lifetime. The aim of this study was to evaluate the effect of four clean label ham formulations (Table 1) (without sodium nitrite and with natural nitrate sources combined with the addition of nitrate reducing cultures) on the human gut microbiota of potential consumers after in vitro digestion, according to the INFOGEST protocol, and colonic fermentation.

Table 1. Ham formulations.

Code	Composition
A	Commercial standardised natural nitrate-rich powder + Meat culture 1
B	Commercial standardised natural nitrate-rich powder + Meat culture 2
C	Blanching water from vegetables + Meat culture 1
D	Blanching water from vegetables + Meat culture 2
E	Control with the nitrifying salt (150 mg kg^{-1} sodium nitrite)

METHODS

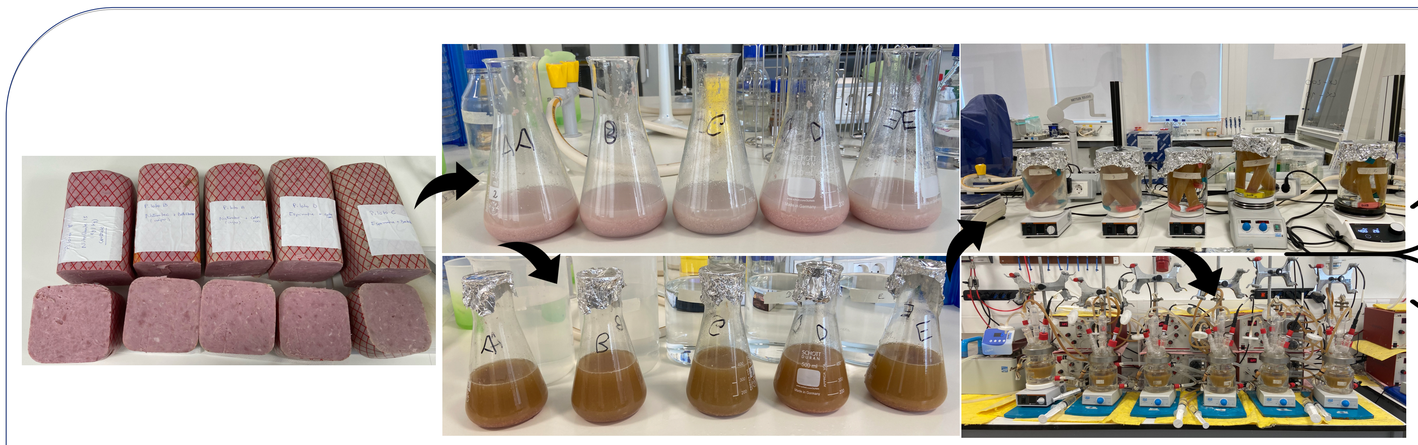
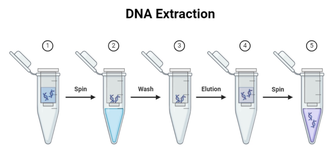


Figure 1. in vitro digestion and colonic fermentation following the INFOGEST protocol.

DNA extraction from the stored pellets was carried out using the Invitrogen PureLink™ Microbiome DNA Purification Kit. 16S rRNA gene sequence data was processed with QIIME2 by Novogene UK;



Quantification of fermentation metabolites, short-chained fatty acids and branched-chained fatty acids, by high-performance liquid chromatography (HPLC) from supernatant.

- The impact of each novel formulation and for a faecal inoculum control (Inoc) on the gut microbiota profile and fermentation metabolites (i.e., SCFAs) was assessed by next-generation sequencing (NGS) and high-performance liquid chromatography (HPLC), respectively.

Results and discussion

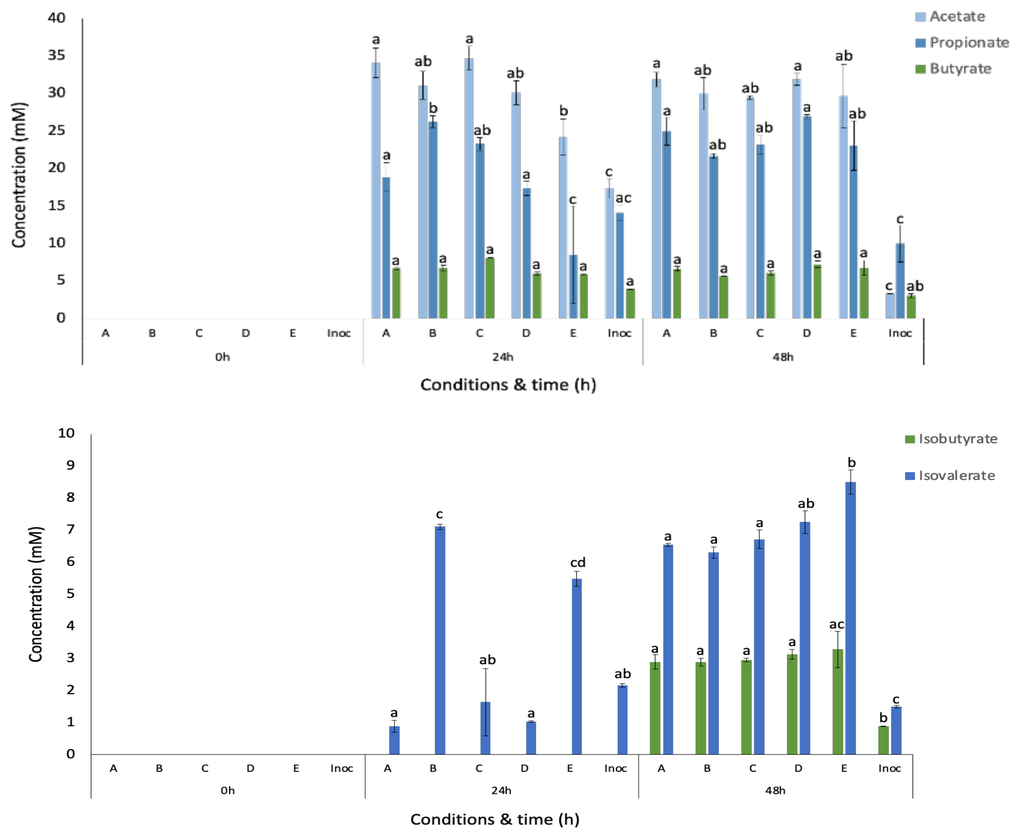


Figure 2. Concentration (mM, means ± SD) of SCFA and BCFA during colonic fermentation.

- Higher concentration of SCFA (Acetate + Butyrate + Propionate) after 48 h colonic fermentation were found for: D (65.96 mM) > A (63.36 mM) > E (59.35 mM) > C (58.64 mM) > B (57.22 mM) > Inoc (16.20 mM);
- Lowest SCFA concentrations were found for the inoculum control (Inoc), as expected, due to the lack of nutrients to promote bacterial fermentation over time.

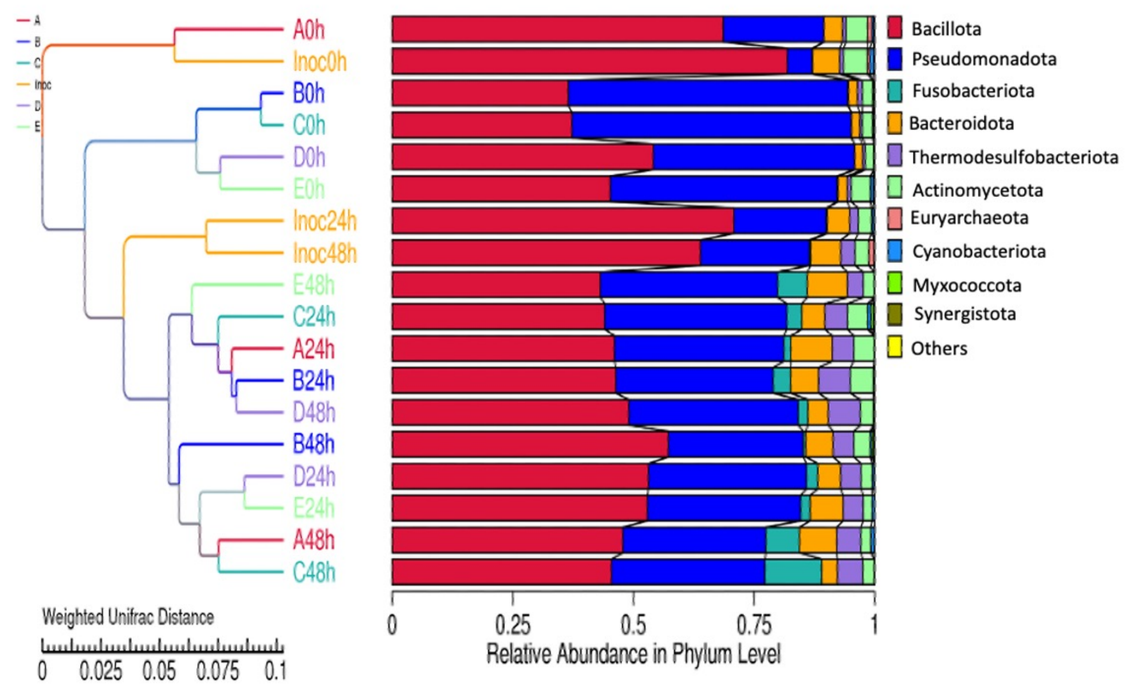


Figure 3. Changes in relative abundance during colonic fermentation (UPGMA cluster tree based on weighted Unifrac distance).

- The most abundant phylum present were *Bacillota* and *Pseudomonadota* for all samples (A, B, C, D, E and Inoc) and all time points (0h, 24h and 48h);
- Higher relative abundance of *Pseudomonadota* was observed for all samples when compared to the inoculum control (Inoc);
- After fermentation, relative abundance of *Bacteroidota* is higher for the ham control sample (added sodium nitrite) than for the samples that are formulated with natural plant nitrate coupled with starter cultures.

Conclusions

- There were no discernible variations in SCFA levels or microbial populations during colonic fermentation between the new formulations and the conventional ham, indicating that the suggested clean label approach produced encouraging outcomes;
- Additional investigation should provide light on the endogenous production of volatile and non-volatile chemicals (nitrosamines) in vitro, their effects on the microbiota in the human gut, and any potential toxicity to human intestinal epithelial cells.

Acknowledgements

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