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Title:

In Vitro Fermentation of Insoluble Date Fruit (*Phoenix dactylifera* L.) Fibre-Enriched Pea and Soy Protein-Based Meat Analogues: Fibre-Driven Modulation of Gut Microbiome and Metabolic Activity

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Preparation of Your Abstract

1. The title should be as brief as possible but long enough to indicate clearly the nature of the study. Capitalise the first letter of the first word ONLY (place names excluded). No full stop at the end.
2. Abstracts should state briefly and clearly the purpose, methods, results and conclusions of the work.
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Dietary fibres (DFs) are critical for enhancing the nutritional and functional properties of plant-based meat analogues. This study investigates the impact of insoluble date fruit fibres (0%, 10%, and 20% w/w) on gut microbiota activity and metabolic outputs using pea protein isolate (PPI)- and soy protein isolate (SPI)-based high-moisture meat analogues (HMMAs).

PPI- and SPI-based HMMAs were digested using the INFOGEST 2.0 protocol, followed by 48-hour in vitro fecal fermentation. Fermentation supernatants were analyzed for pH, short-chain fatty acids (SCFAs: acetate, propionate, butyrate), gas production, reducing sugars, and free fatty acids via HPLC-UV and spectrophotometric assays. Microbial DNA was extracted for 16S rRNA sequencing (V3–V4 regions) to assess taxonomic composition and functional pathways (QIIME2, LEfSe, PICRUSt2).

Fibre concentration consistently modulated microbial activity in both PPI and SPI fermentation systems. Increased dietary fibre levels led to enhanced pH reduction and SCFA production, particularly butyrate, with 20% fibre formulations (PPE20 and SPE20) showing the most pronounced effects. Taxonomic shifts revealed substantial enrichment of key butyrate-producing bacteria, including *Faecalibacterium duncaniae* (3.2× in PPE20), Dorea longicatena (10.9× in PPE20), and *Collinsella aerofaciens* (11.3× in PPE20), relative to baseline. These changes were accompanied by a decline in proteolytic taxa such as *Morganella morganii* in SPE20, suggesting fibre-driven suppression of potentially pro-inflammatory microbes. Gas production and reducing sugar depletion patterns further supported fibre-induced shifts in microbial metabolism, highlighting the role of fibre in promoting beneficial SCFA-producing communities regardless of protein source.

This work demonstrates that insoluble date fibres act as potent bioactive agents in plant-based meats, with dose-dependent effects on gut microbiome composition and metabolic activity. The findings highlight the broader applicability of fibre enrichment in diverse protein systems to advance gut-health-optimized plant-based foods.