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Title:

Microbiome transmission through simulated spinach digestion and modulatory effects of olive oil dressing

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Abstract: (Your abstract must use **Normal style** and must fit in this box. Your abstract should be no longer than 300 words. The box will 'expand' over 2 pages as you add text into it.)

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.

Introduction: Clearly state the purpose of the abstract
Methods: Describe your selection of observations or experimental subjects clearly
Results: Present your results in a logical sequence
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Fresh plant-based produce constitutes a major dietary source of diverse live microbiota, often harbouring billions of organisms per gram. Occasionally these include pathogenic contaminants such as *Escherichia coli* O157:H7 and *Salmonella Typhimurium*, known to survive human digestion, colonise the gut, and cause disease. However, little is known about whether the broader produce-associated microbiota persist through digestion, or how simple dietary practices may modulate this.

This study employed spinach as a model edible microbiome system and olive oil as a representative salad dressing component used in common dietary practice. Specifically, we aimed to: (1) evaluate survival of produce-associated microbiota and common foodborne pathogens during simulated human digestion; (2) assess whether olive oil alters microbial persistence; and (3) explore whether dietary microbes influence the production of bioactive secondary bile acids (SBAs) in the gut in the presence and absence of olive oil (metabolomic analyses ongoing).

Spinach was spiked with *E. coli* O157 and *S. Typhimurium* and subjected to simulated digestion using the standardised INFOGEST model, with and without olive oil dressing. Microbial persistence was assessed via culture-based quantitation and PacBio full-length 16S rRNA sequencing, while selected samples were retained for prospective SBA metabolomic profiling.

Results showed that a substantial fraction of the spinach microbiome persisted throughout digestion, supporting the notion of a digestion-resistant microbiome and potential food-gut microbiome continuum. Pathogen concentrations remained stable throughout all digestion phases, with no significant impact of olive oil addition. Conversely, olive oil differentially modulated the persistence of remaining non-pathogenic spinach taxa.

This study provides novel insights into produce-to-gut microbiome transmission. Although olive oil did not affect *E. coli* O157 or *S. Typhimurium* survival, it selectively impacted persistence of other microbiota, highlighting how everyday dietary practices may modulate food–gut microbial transmission. Ongoing metabolomic analyses will help elucidate whether such shifts may influence downstream gut metabolic interactions.