

## Effect of fibre combination on gas production by human gut bacteria *in vitro*

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Most dietary fibres can be fully or partially fermented by commensal microbiota in the colon, releasing beneficial bioactive molecules<sup>(1)</sup>. However, the production of gas during the rapid fermentation of fibres may cause undesirable symptoms for some people<sup>(2)</sup>. Dietary exposure to highly soluble fermentable fibres used in food reformulation, such as inulin and fructo-oligosaccharides, is increasing and the impact this may have on digestive symptoms is unclear. The aim of this study was to investigate the *in vitro* fermentation of three highly soluble fermentable fibres (inulin (In), short chain fructo-oligosaccharide (scFOS) and soluble corn fibre (SCF)) individually, and in combination with one another. Outcome measures were gas production and pH change.

Healthy adults (18–65 y/o) (n = 12) provided a stool sample used to inoculate an *in vitro* colonic fermentation model<sup>(3)</sup>. A faecal slurry (16% w/v) was prepared and added to a fermentation bottle (13 per fermentation; duplicates for each fibre/fibre combination and a no fibre control) along with fermentation medium and 1 g total fibre substrate. Bottles were incubated in a shaking water bath at 37°C for 24 h. Aliquots of fermentation slurry were collected at baseline (0), 4, 8 and 24 h for pH measurement. Gas production was measured at 4, 8 and 24 h. Ethical approval was granted by the University of Glasgow College of Medical, Veterinary and Life Sciences Ethics Committee (Project no. 200190107).

The pH (mean (SD)) of all fibre conditions was significantly lower than the control at 24 h ( $p < 0.01$ ) (In: 4.4 (0.2), scFOS: 4.2 (0.1), SCF: 5.1 (0.2), In + scFOS: 4.2 (0.1), In+SCF: 4.3 (0.1), scFOS+SCF: 4.1 (0.1), control: 7.4 (0.1)). There was considerable inter-individual variation in total gas production ((ml) median (IQR)) (In: 40.8 (43.4); scFOS: 35.8 (23.4), SCF: 38.3 (28.8), In+scFOS: 40.5 (28.6), In+SCF: 32.3 (20.5), scFOS+SCF: 32.3 (31.4)). At all timepoints pH was significantly correlated with gas production (4h:  $r = -0.751$ , 8h:  $r = -0.430$ , 24 h  $r = -0.262$  ( $p < 0.01$ )). By 24 h pH differed between the fibre conditions, suggesting fermentation rate may have varied. Gas production across the fibre conditions was highly variable between individual sample donors despite all substrates being highly fermentable. Gas production was consistently higher in the samples from some donors, whereas others produced very little e.g. total cumulative gas production with In was 24 ml from one donor sample and 273.5 ml for another. Gas production rate from different fibres seemed characteristic to each individual. Gas volume in the colon is dynamic and is determined by both production and utilisation by the commensal microbiota (e.g. CO<sub>2</sub> into acetate). Differences in substrate preference and composition of gut microbiota between individuals may explain some of the inter-individual variation we observed in this study. More fermentations would be needed to identify differences in gas production between the different fibre conditions.

### References

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