

LETTERS

Water activity does not shape the microbiota in the human colon

We recently reported an association between stool consistency as measured by the Bristol Stool Scale (BSS) and major markers of the gut ecosystem, including microbial richness, enterotypes and bacterial growth rates in 53 healthy women.¹ Meanwhile, the link between stool consistency and colon microbiota composition has been confirmed in two large-scale ($n > 1000$) cohorts including individuals of both sexes, different age and varying health statuses.^{2,3} In our original manuscript, we hypothesised on mechanisms that would explain the associations observed. Two potential mechanisms were put forward, namely colon ecosystem differentiation through passage rate variation and reduction of water availability. While the impact of passage rate on ecosystem composition has recently been shown to partially account for BSS-associated microbiota variation,⁴ the potential correlation with water availability remains unexplored.

Water activity (a_w) is a dimensionless variable that reflects the availability of water in a system (a_w pure water = 1). It differs from water moisture in the sense that the latter quantifies both molecular-bound and freely available water. The relation between water activity and moisture content is non-linear and product specific and temperature specific, but has yet to be determined for faeces. Water activity is essential for enzymatic reactions, thus making it a key factor of selective pressure on microbial growth.⁵ Its relevance in a driving force for gut ecosystem compositional variation is currently unclear.

Here, we measure water activity of 62 fresh faecal samples with different consistencies using a resistive electrolytic hygrometer (LabMaster, Novasina, Lachen, Switzerland; accuracy 0.003 a_w ; technical variability 0.002 a_w), which measures relative air humidity through the resistance of an electrolyte at constant temperature (37°C). We show that water activity in faeces correlates with neither moisture content (Spearman $r = 0.12$, p value > 0.05 ; figure 1A) nor BSS scores (Spearman $r = 0.03$, p value > 0.05 ; figure 1B). Despite large differences in moisture content, which correlate well with BSS scores (Spearman $r = 0.52$, p value < 0.001 ; figure 1C), water activity of fresh

faecal samples displays little variation (table 1). The values recorded are consistently higher than 0.97 a_w (table 1).

Given the relevance of water activity in the context of food spoilage, there is ample availability of data regarding minimal a_w values required to allow the

growth of specific micro-organisms. From these reports, it is clear that a_w above 0.97 provide sufficient unbound water to support the growth of most bacteria.⁶ As a_w values of fresh faecal samples can be expected not to exceed those in the colon environment, our results indicate that

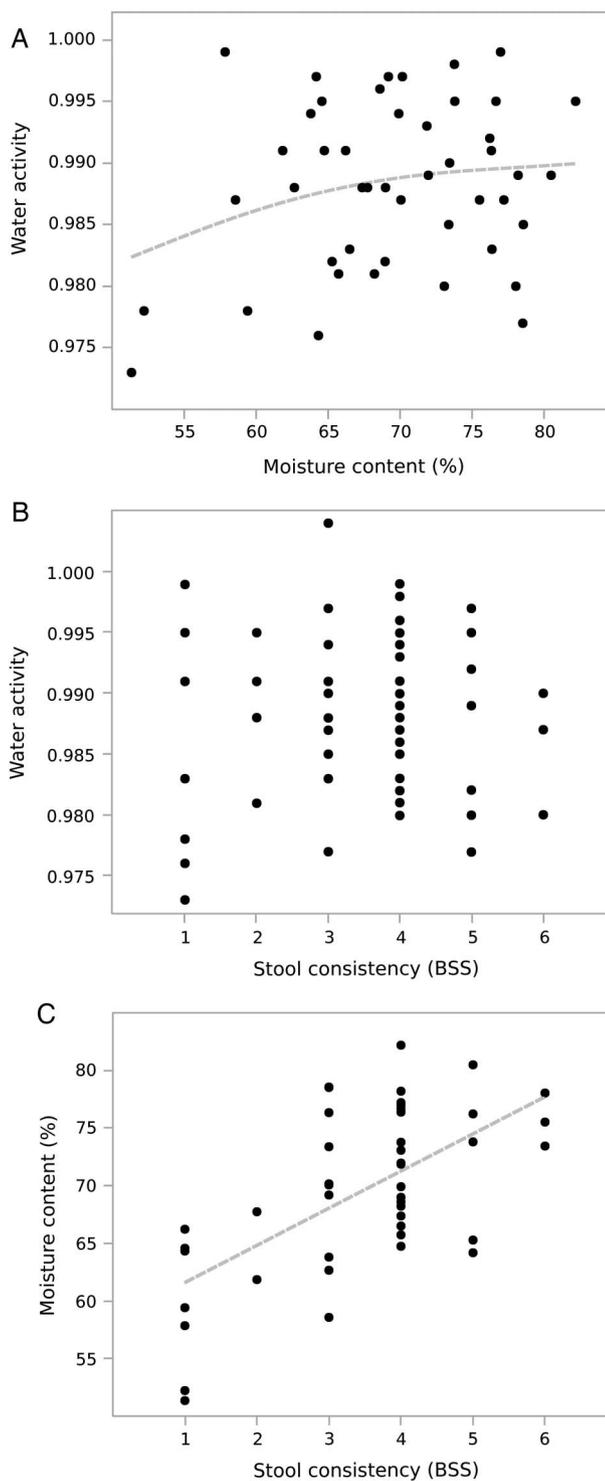


Figure 1 (A) Moisture sorption isotherm curve for faeces (Spearman $r = 0.12$, p value > 0.05). **(B)** Correlation between water activity and Bristol Stool Scale (BSS) scores (Spearman $r = 0.03$, p value > 0.05). **(C)** Correlation between moisture content and BSS scores (Spearman $r = 0.52$, p value < 0.001).

Table 1 Data summary

	All (62)	Women (32)	Men (30)
Stool consistency (BSS)	1–6 (4)	1–6 (3)	1–6 (4)
Moisture content (%)	51.35–82.19 (69.55)	51.35–80.49 (68.6)	52.22–82.19 (71.86)
Water activity	0.973–1.004 (0.9875)	0.973–1.004 (0.9875)	0.977–0.999 (0.9875)

The values are expressed in minimum–maximum (median). BSS, Bristol Stool Scale.

water activity variation in the colon is too limited to exert any selective pressure on the residing bacteria. The observed effects of stool consistency on colon microbiota composition are thus unlikely to be a result of water activity variation, but rather represent transit time differences or other, currently unassessed variables.

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Data sharing statement Water activity and moisture content data are made available on request to the corresponding author.



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