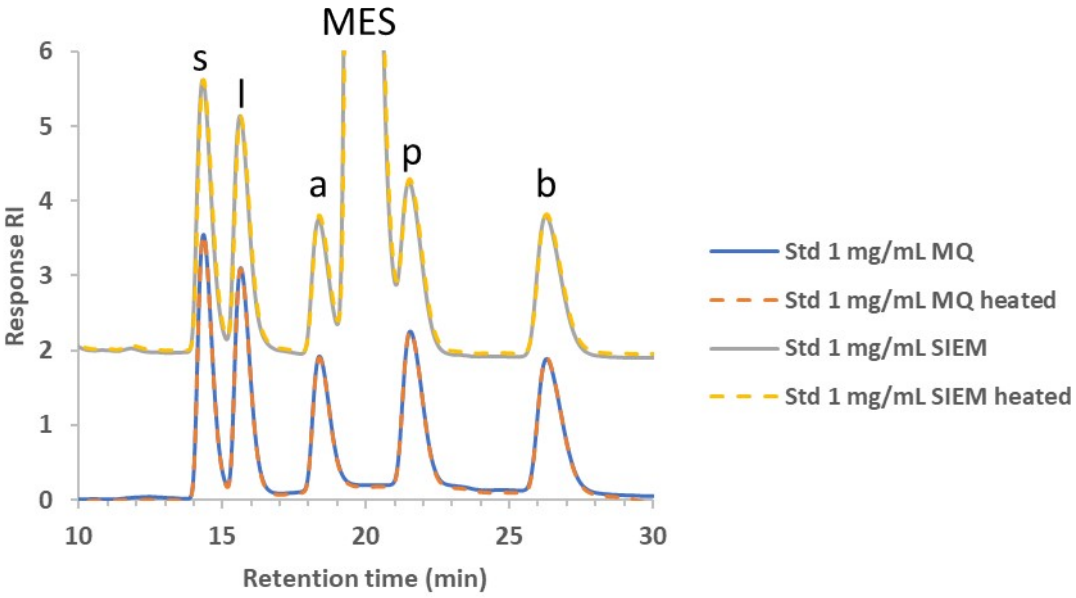
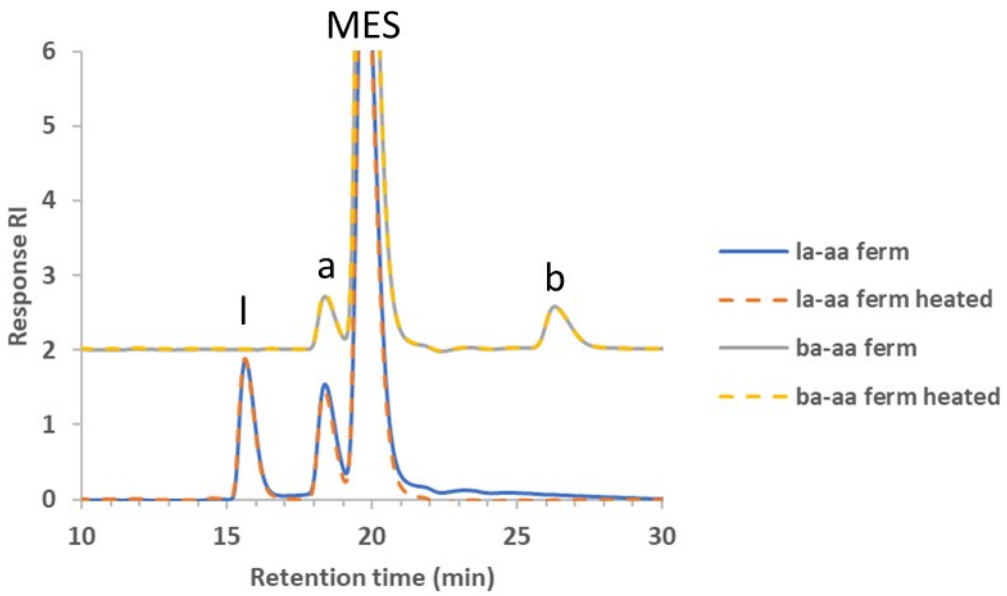
Supplementary Figures: Validation of heating treatment of digesta with respect to SCFA analysis.

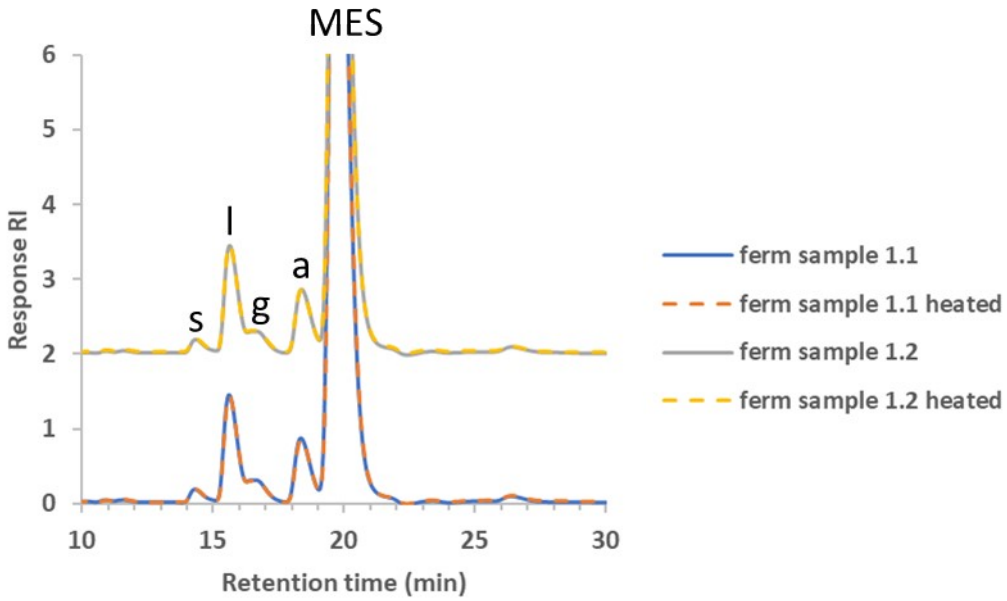
To exclude that heating of digesta to inactivate enzymes and to kill bacteria had any effect on the quantification of SCFAs, a standard of 1 mg/mL of each acid was prepared, both in milli-Q water and in the medium used for the experiment (SIEM) (Supplementary Figure 1). Half of the standard was heated to 100 °C for 10 min in a safe-lock Eppendorf tube, subsequently cooled down to room temperature, centrifuged, tube opened (at temperature equal or lower to room temperature) after which the supernatants were analyzed using HPLC-UV-RI. The other part was non-heated, centrifuged, filtered and analyzed on HPLC-UV-RI. Next to these standards, we also included samples that contained a similar concentration as after fermentation of some substrates (Supplementary Figure 2). Additionally, we analyzed some fermentation samples with the same method (Supplementary Figure 3 & 4). Our conclusion is that the elution patterns completely overlap and that none of the acids present in the various samples/mixes showed any lower concentration due to the heating step.



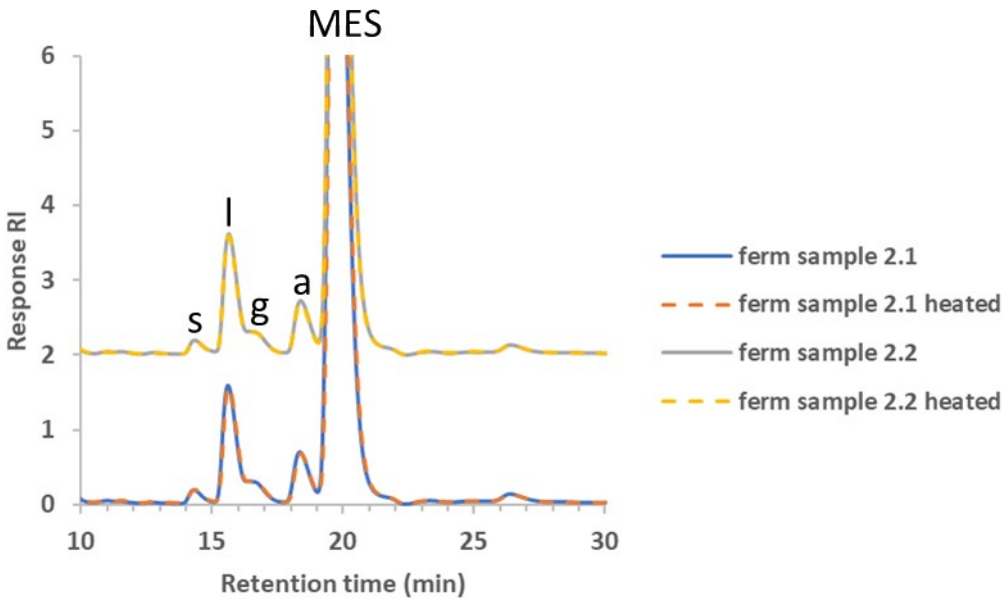
Supplementary Figure 1. SCFA and organic acid analyzed using HPLC-RI-UV. Standards of 1 mg/mL of each acid were prepared in MQ or in SIEM medium. Half was heated and analyzed, the other part was unheated and analyzed. S, l, a, MES, p, b refers to succinate, lactate, acetate, MES buffer, propionate and butyrate, respectively.



Supplementary Figure 2. SCFA and organic acid analyzed using HPLC-RI-UV. SCFA concentrations as could be present after fermentation of different substrates in SIEM medium. Half of the sample was heated and then analyzed, the other part of the sample was unheated and directly analyzed. L, a, MES, b refers to lactate, acetate, MES buffer and butyrate, respectively. La=lactic acid; aa=acetic acid; ba=butyric acid; Ferm=concentration of SCFA mimicking concentrations as present in real fermentation samples.



Supplementary Figure 3. SCFA and organic acid analyzed using HPLC-RI-UV after 48 h of *in vitro* fermentation of substrate 1 using 6-month-old infant inoculum. Half of the fermentation broth was heated and analyzed, the other part was unheated and analyzed. S, l, g, a and MES refers to succinate, lactate, glycerol, acetate and MES buffer, respectively.



Supplementary Figure 4. SCFA and organic acid analyzed using HPLC-RI-UV after 48 h of *in vitro* fermentation of substrate 2 using 6-month-old infant inoculum. Half of the fermentation broth was heated and analyzed, the other part was unheated and analyzed. S, l, g, a and MES refers to succinate, lactate, glycerol, acetate and MES buffer, respectively.