

Supplementary material

IBS randomized study: FODMAPs alter bile acids, phenolic- and tryptophan metabolites,
while gluten modifies lipids

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Supplementary Text 1.

Parameters in XCMS

Reversed phase chromatography – Negative ionization (RN)

Peak picking:

```
CentWaveParam(peakwidth=c(8.2,53.9), noise=500, snthresh=10, ppm=17.6, mzdif=0.0021,  
prefilter=c(3,3500), integrate=1)
```

Retention time correction:

```
PeakDensityParam(minfrac=0.95, bw=1, binSize=0.02) peakGroupParam(minfrac=0.95,  
smooth="loess", span=0.4, family="gaussian")
```

Correspondence:

```
PeakDensityParam(minfrac=0.4, bw=1, binSize=0.02); FillChromPeaksParam(ppm=19,  
fixedRt=0.25, expand=0.1)
```

Reversed phase chromatography – Positive ionization (RP)

Peak picking:

```
CentWaveParam(peakwidth=c(8.5,52), noise=500, snthresh=10, ppm=18.3, mzdif=0.0019,  
prefilter=c(3,2500), integrate=1)
```

Retention time correction:

```
PeakDensityParam(minfrac=0.95, bw=1, binSize=0.02)  
peakGroupParam(minfrac=0.95, smooth="loess", span=0.4, family="gaussian")
```

Correspondance:

```
PeakDensityParam(minfrac=0.4, bw=1, binSize=0.02); FillChromPeaksParam(ppm=20, fixedRt=1,  
expand=0.1)
```

Parameters used for RamClust

Parameters were manually optimized by visual inspection of extracted ion chromatograms of 20 randomly selected cluster, resulting in $st = 1.25$, $sr = 0.35$ in both modes. This resulted in 1018 clusters in RN and 1377 in RP and 2784 and 3439 singletons, respectively. For downstream analysis, the feature with highest intensity in each cluster was selected together with the singletons.

Supplementary Table 1. Selected metabolites from random forest modelling of the FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) versus placebo models that were not possible to identify. Data is analysed with linear mixed models and presented as overall p-value in ANOVA, as well as pairwise fold change (FC) and p-value between interventions, with n=100 and one additional missing value for the FODMAP intervention.

mz_rt ^a	Molecular formula	Level ^{b,c}	ANOVA p-value	Fold change and p-value		
				FODMAPs- Placebo	FODMAPs- Gluten	Gluten- Placebo
RP200.071749786501_129.674144197569		5		FC= 2.23, p<0.0001	FC= 2.22, p<0.0001	FC= 1.01, p=1 FC=
RN469.225621819326_374.754534346797		5	p<0.0001	FC= 0.79, p<0.0001	FC= 0.78, p<0.0001	1.02, p=0.6 FC=
RN231.033027186211_273.585695459787		5	p=0.005	FC= 0.77, p=0.002	FC= 0.91, p=0.3	0.84, p=0.04 FC=
RP1083.78856079003_484.898312142648		5	p=0.004	FC= 1.08, p=0.001	FC= 1.04, p=0.07	1.04, p=0.1 FC=
RN141.867754527625_200.368515375446		5	p=0.02	FC= 0.96, p=0.01	FC= 0.96, p=0.03	0.99, p=0.7 FC=
RP310.165030574733_158.787871676796		MS1	p<0.0001	FC= 1.54, p<0.0001	FC= 1.31, p=0.001	1.18, p=0.05 FC=
RN605.155287981323_404.923402871319		MS1	p=0.0009	FC= 1.06, p=0.003	FC= 1.07, p=0.0008	0.99, p=0.7 FC=
RN307.027729303265_258.761685388328		5	p=0.03	FC= 0.77, p=0.02	FC= 0.78, p=0.03	0.98, p=0.9 FC=
RP233.107096562762_276.257581395272		5	p=0.0002	FC= 1.11, p<0.0001	FC= 1.03, p=0.3	1.08, p=0.003 FC=
RN183.016060389807_53.6153560961997		MS1	p=0.02	FC= 1.05, p=0.008	FC= 1.04, p=0.03	1.01, p=0.6 FC=
RN400.223997796269_391.752567409242	C ₁₉ H ₃₅ N ₃ O ₄ S	4	p=0.006	FC= 0.75, p=0.002	FC= 0.89, p=0.2	0.85, p=0.06 FC=
RP157.006851795851_102.92335384128		MS1	p=0.006	FC= 0.88, p=0.002	FC= 0.95, p=0.2	0.93, p=0.07 FC=
RN1014.56315855689_569.822826515375		5	p=0.0007	FC= 1.08, p=0.0009	FC= 1.07, p=0.001	FC= 1, p=0.9 FC=
RN162.839201406487_200.444004178418		5	p=0.0006	FC= 0.93, p=0.0002	FC= 0.95, p=0.01	0.97, p=0.2 FC=
RP294.046323101249_200.782608969917		MS1	p=0.0002	FC= 0.86, p=0.0002	FC= 0.88, p=0.001	0.98, p=0.7 FC=
RP88.0755974397113_105.944971285564		5	p<0.0001	FC= 1.15, p=0.002	FC= 1.21, p<0.0001	0.95, p=0.2 FC=
RN235.170116960809_387.616139937925		5	p=0.01	FC= 1.03, p=0.004	FC= 1.01, p=0.2	1.02, p=0.1 FC=
RN568.290324797654_507.438889561808		MS1	p=0.006	FC= 1.15, p=0.003	FC= 1.02, p=0.6	1.13, p=0.01

mz_rt ^a	Molecular formula	Level ^{b,c}	ANOVA p-value	Fold change and p-value		
				FODMAPs- Placebo	FODMAPs- Gluten	Gluten- Placebo
RN523.930304435419_404.592342682707		5	p=0.01	FC= 0.91, p=0.005	FC= 0.94, p=0.05	FC= 0.97, p=0.4 FC=
RN232.028359519673_87.1592109106064		5	p=0.07	FC= 0.86, p=0.02	FC= 0.94, p=0.4	0.91, p=0.2 FC=
RP164.034404926688_148.389892575338		MS1	p<0.0001	FC= 0.83, p=0.0001	FC= 0.85, p=0.0004	0.98, p=0.7 FC=
RN428.174575687692_317.773992843601		5	p=0.1	FC= 0.83, p=0.05	FC= 0.98, p=0.8	0.85, p=0.09 FC=
RN811.204608324584_478.508451416692		MS1	p=0.09	FC= 1.03, p=0.06	FC= 1, p=1	1.03, p=0.06 FC=
RN269.838475694601_200.295489034855		5	p=0.01	FC= 0.91, p=0.004	FC= 0.94, p=0.09	0.96, p=0.2 FC=
RN273.043450678584_257.740295012614		5	p=0.06	FC= 0.73, p=0.03	FC= 0.77, p=0.06	0.95, p=0.7 FC=
RN660.843693308895_430.883484405387		MS1	p=0.003	FC= 0.74, p=0.0008	FC= 0.85, p=0.06	0.88, p=0.1 FC=
RP254.01576735935_200.635217350717		MS1	p<0.0001	FC= 0.81, p<0.0001	FC= 0.83, p=0.0005	0.97, p=0.6 FC=
RN121.029619427165_157.250314773886	C ₇ H ₆ O ₂	4	p<0.0001	FC= 1.29, p<0.0001	FC= 1.26, p<0.0001	1.02, p=0.6 FC=
RN162.019460020307_148.89233990703		5	p=0.007	FC= 0.93, p=0.002	FC= 0.96, p=0.06	0.97, p=0.2 FC=
RP205.058939915497_42.3977618960263		5	p<0.0001	FC= 0.79, p<0.0001	FC= 0.8, p<0.0001	0.98, p=0.8 FC=
RN1205.2679954259_454.975961354632		5	p=0.08	FC= 0.99, p=0.7	FC= 1.02, p=0.09	0.97, p=0.03 FC=
RP751.601119591421_444.679510503933		MS1	p=0.02	FC= 0.92, p=0.01	FC= 0.99, p=0.7	0.93, p=0.03 FC=
RN237.149613010517_389.083501247032		5	p=0.02	FC= 0.91, p=0.008	FC= 0.99, p=0.7	0.92, p=0.02 FC=
RN241.069073705849_104.663135022765		5	p=0.007	FC= 0.9, p=0.003	FC= 0.92, p=0.02	0.98, p=0.6

^aThe column mz_rt shows if data were analyzed in positive mode, “RP” or negative mode “RN”, thereafter mass to charge (mz) and retention time (rt) value are presented separated by “_”

^bLevel of identification is presented as Schymanski’s scale 1-5³²

^cMS1 denotes a lack of recorded MS2

Supplementary Table 2. Selected metabolites from random forest modelling of the gluten versus placebo models that were not possible to identify. Data is analysed with linear mixed models and presented as overall p-value in ANOVA, as well as pairwise fold change (FC) and p-value between interventions, with n=100 and one additional missing value for the FODMAP intervention.

mz_rt ^a	Molecular formula	Level ^{b,c}	ANOVA p-value	Fold change and p-value		
				FODMAPs-Placebo	FODMAPs-Gluten	Gluten-Placebo
RN146.082080095985_52.0277558129751	C ₂₀ H ₃₉ NO ₂ S	MS1		FC= 0.99, p=0.8	FC= 0.89, p<0.0001	FC= 1.12, p<0.0001
RN209.081735723202_282.713239186721		5	p=0.04	FC= 0.99, p=0.8	FC= 0.92, p=0.02	FC= 1.07, p=0.04
RP230.103242750646_334.718744096972		MS1	p=0.004	FC= 0.97, p=0.4	FC= 1.11, p=0.02	FC= 0.87, p=0.002
RP113.034099414214_55.3558471341889		MS1	p=0.004	FC= 1.03, p=0.2	FC= 0.96, p=0.06	FC= 1.07, p=0.001
RP355.172251887188_263.65322618527		4	p=0.003	FC= 1.12, p=0.02	FC= 0.95, p=0.3	FC= 1.18, p=0.001
RP74.0236239949622_53.5586977360521		MS1	p=0.002	FC= 1, p=0.9	FC= 0.89, p=0.003	FC= 1.13, p=0.002
RP198.058810046918_273.159441012274		5	p=0.05	FC= 1.01, p=1	FC= 0.69, p=0.04	FC= 1.46, p=0.03
RP211.094366206553_263.916879329792		MS1	p=0.08	FC= 0.98, p=0.5	FC= 1.04, p=0.1	FC= 0.94, p=0.03
RN1017.22438675431_483.016193466318		MS1	p=0.09	FC= 0.98, p=0.3	FC= 1.02, p=0.3	FC= 0.96, p=0.03
RP341.061849692644_361.663369325829		5	p=0.005	FC= 0.79, p=0.01	FC= 1.06, p=0.5	FC= 0.75, p=0.002
RN380.266227666441_429.33646242798		MS1	p=0.01	FC= 1.04, p=0.2	FC= 0.95, p=0.08	FC= 1.1, p=0.003

^aThe column mz_rt shows if data were analyzed in positive mode, “RP” or negative mode “RN”, thereafter mass to charge (mz) and retention time (rt) value are presented separated by “_”

^bLevel of identification is presented as Schymanski’s scale 1-5³²

^cMS1 denotes a lack of recorded MS2

Supplementary Table 3. Main MS/MS fragments of identified features.

mz_rt ^a	Identification	MS/MS fragmentation
RN188.071688028145_267.375085019106	3-indolepropionic acid	59.0133, 116.0500, 144.0816, 188.0716
RN165.055390498355_219.297269460009	3-(3-Hydroxyphenyl)propanoic acid	106.0428, 119.0505, 121.0662, 165.0561
RN194.045963036146_131.780009747153/ RN194.045998607944_145.671676422607	3-Hydroxyhippuric acid	93.0344, 150.0557, 194.0456
RN274.00218258369_105.441834500593/ RN274.002088122407_123.125213172007	Hippuric acid metabolite	93.0352, 150.0553, 194.0459, 274.0011
RN391.285138794537_377.086763193047	Hyodeoxycholic acid ^b	391.2851
RP100.075529281117_121.06338078176	2-piperidione	44.0132, 56.0497, 82.0657, 100.0752
RP61.0395495784532_43.1850182223804	Urea	44.0127, 61.0394
RP262.16420935626_55.3955350224906/ RP262.164500075168_91.9418173807388	3-Hydroxyisovalerylcarbitine	59.0484, 85.0283, 262.165

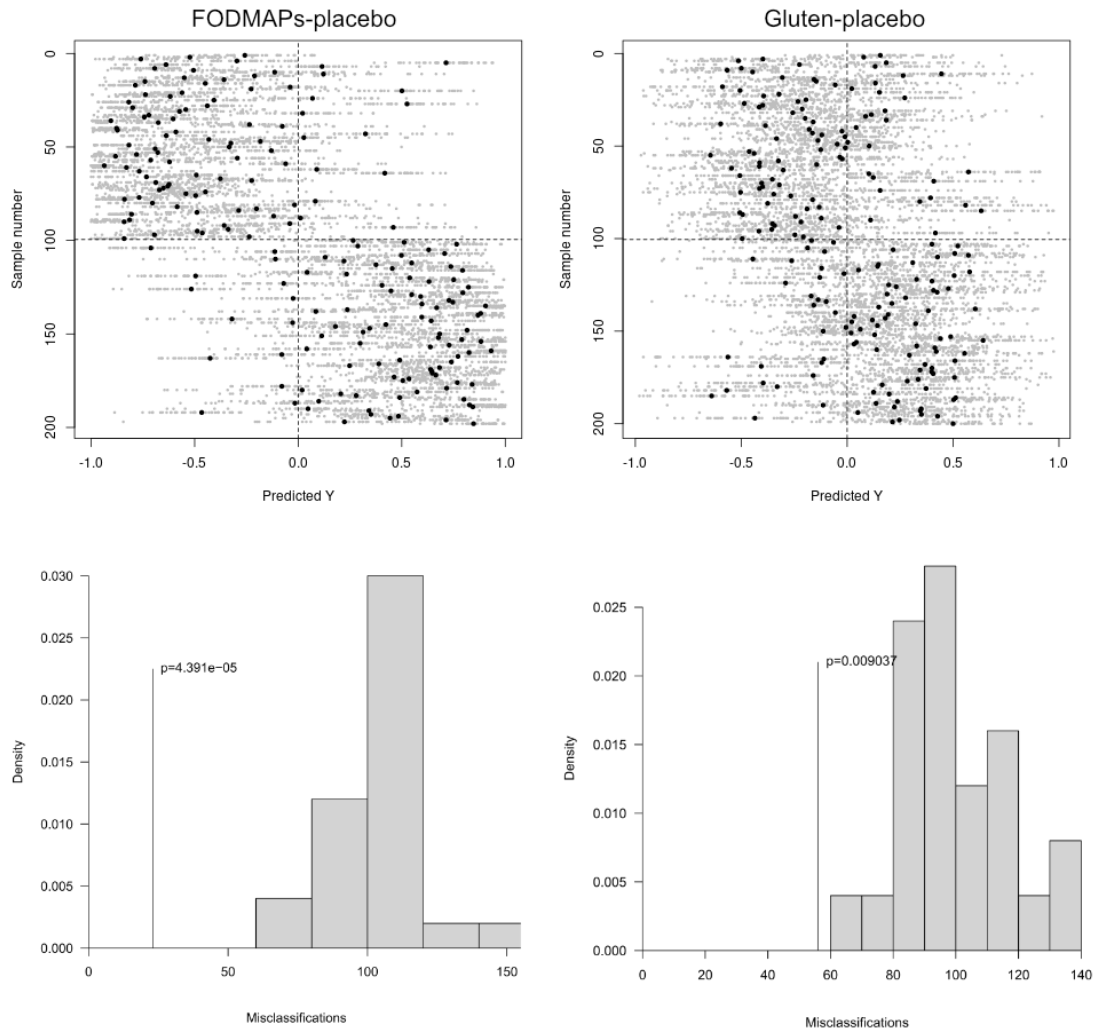
^aThe column mz_rt shows if data were analyzed in positive mode, “RP” or negative mode “RN”, thereafter mass to charge (mz) and retention time (rt) value are presented separated by “_”

^bThe feature was neither fragmented in the standard nor the sample. However, several standards were run matching the mz; deoxycholic acid, ursodeoxycholic acid, chenodeoxycholic acid, and hyodeoxycholic acid. Hyodeoxycholic acid had a perfect match in rt and a similar behaviour of no fragmentation of the feature, verifying the identity

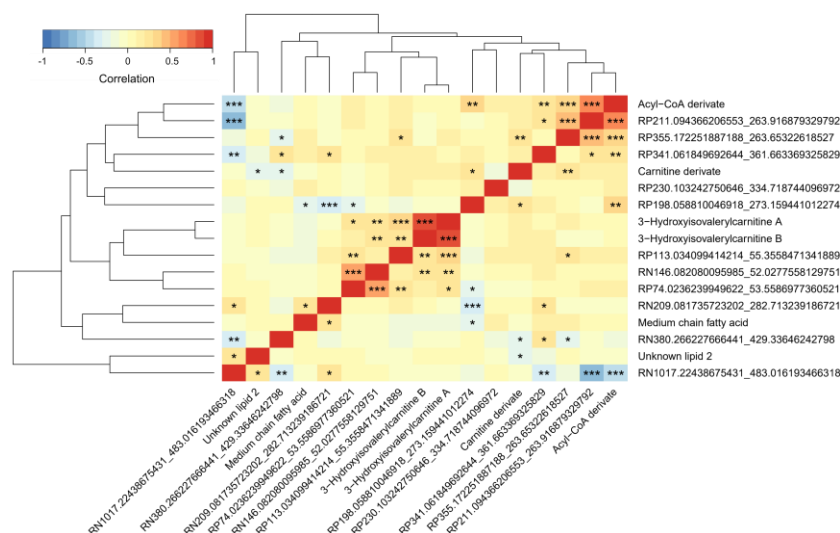
Supplementary Table 4. Standards run for failed attempts of identification.

mz_rt ^a	Standard
RP130.064730227491_265.680809142092	3-(Dimethylaminomethyl)indole, Quinoline
RP132.080668053373_242.94311900296	Skatole
RP146.060079484304_203.428347969286	Indole-3-carboxaldehyde
RP274.200784753243_222.663472116277	Heptanoylcarbitine
RN121.029619427165_157.250314773886	Benzoic acid
	2-Hydroxybenzaldehyde, 4-Hydroxybenzaldehyde, trans-3-(2-Furyl)acrolein
RN159.102571268848_305.238949041253	(3S)-3-Hydroxyoctanoic acid
RN446.290757856995_355.889625521654	Glycocholic acid

^aThe column mz_rt shows if data were analyzed in positive mode, “RP” or negative mode “RN”, thereafter mass to charge (mz) and retention time (rt) value are presented separated by “_”

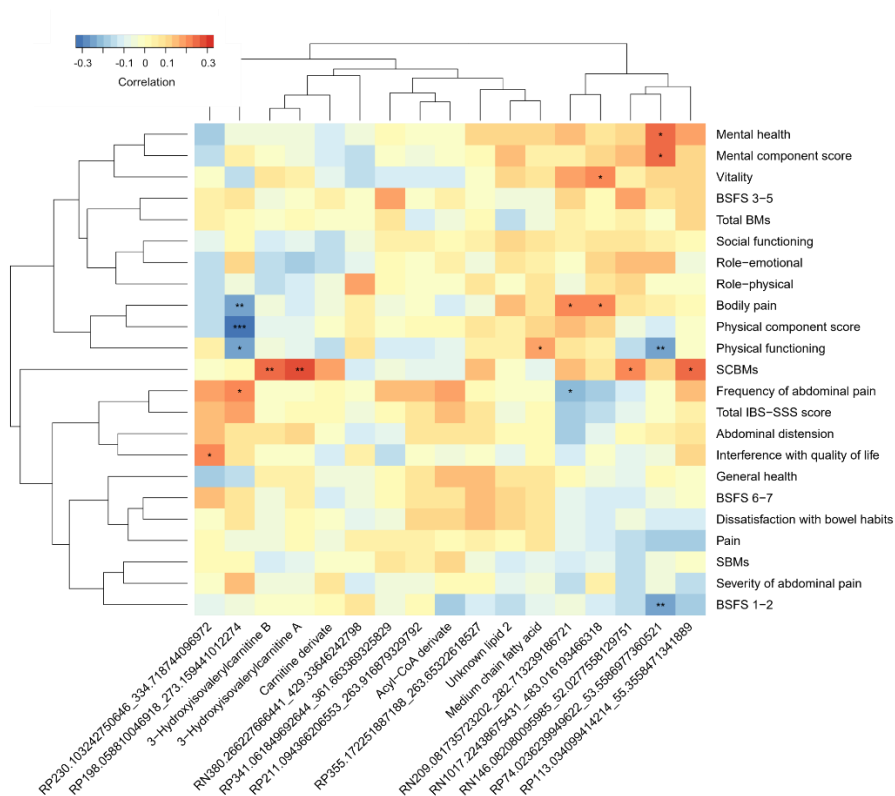


Supplementary Figure 1. Due to sample dependency related to the crossover design, random forest modelling was performed as multilevel analysis, plots are presented in the top row based on the models FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) versus placebo and gluten versus placebo, with clear separation for the FODMAP versus placebo model, indicating on a marked difference in the metabolome between the interventions, meanwhile there were a modest effect of the gluten intervention. To assure results were not due to chance findings due to overfitting, permutation test was performed and presented in the bottom row, confirming robust results.



Supplementary Figure 2. Partial spearman correlation and identified clusters of gluten-related metabolites from random forest modelling for the gluten versus placebo model, adjusted for age and sex (n=100).

P-values <0.05 presented with the star system * = p<0.05, ** = p<0.01, *** = p<0.001



Supplementary Figure 3. Partial spearman correlation between metabolites selected from random forest modelling for the gluten versus placebo model and the questionnaires IBS-SSS, the bowel diary and the health and wellbeing questionnaire (SF-36v2), adjusted for age and sex, n=100.

* = p<0.05, ** = p<0.01, *** = p<0.001

Abbreviations: BM, bowel movements; BSFS, Bristol stool form scale; Irritable Bowel Syndrome- Severity Scoring System; SBMs, spontaneous bowel movement; SCBMs, spontaneous complete (a sensation of complete evacuation) bowel movement; Pain, abdominal pain associated with bowel emptying